Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	5111	carbon near2 (flux or flow)	US-PGPUB; USPAT	OR	OFF	2005/05/13 15:33
L2	374	1 near4 (modif$8 or alter$8 or increas$8)	US-PGPUB; USPAT	OR	OFF	2005/05/13 15:33
L3	233	(phosphoenol adj pyruvate or pep or phosphoenolpyruvate or phospho adj enol adj pyruvate) near4 (suppl$4 or availab$8)	US-PGPUB; USPAT	OR	OFF	2005/05/13 15:34
L4	11	2 and 3	US-PGPUB; USPAT	OR	OFF	2005/05/13 15:34
L5	8702	phosphotransferase$1 or phospho adj transferase$1	US-PGPUB; USPAT	OR	OFF	2005/05/13 15:51
L6	58	(2 or 3) and 5	US-PGPUB; USPAT	ADJ	OFF	2005/05/13 15:51
L7	43	(2 or 3) same (aromatic or shikimate)	US-PGPUB; USPAT	OR	OFF	2005/05/13 16:07

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	5111	carbon near2 (flux or flow)	US-PGPUB; USPAT	OR	OFF	2005/05/13 15:33
L2	374	1 near4 (modif$8 or alter$8 or increas$8)	US-PGPUB; USPAT	OR	OFF	2005/05/13 15:33
L3	233	(phosphoenol adj pyruvate or pep or phosphoenolpyruvate or phospho adj enol adj pyruvate) near4 (suppl$4 or availab$8)	US-PGPUB; USPAT	OR	OFF	2005/05/13 15:34
L4	11	2 and 3	US-PGPUB; USPAT	OR	OFF	2005/05/13 15:34

PGPUB-DOCUMENT-NUMBER: 20050079617

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20050079617 A1

TITLE: Glucose transport mutants for production of biomaterial

PUBLICATION-DATE: April 14, 2005

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APPL-NO: 10/ 728337

DATE FILED: December 3, 2003

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
WO	PCT/US03/31544	2003WO-PCT/US03/31544	October 3, 2003

US-CL-CURRENT: 435/471

ABSTRACT:

A method is disclosed for restoring a Glu.sup.+ phenotype to a PTS.sup.-/Glu.sup.- bacterial cell which was originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport. Bacterial cells comprising the Glu.sup.+ phenotype have modified endogenous chromosomal regulatory regions which are operably linked to polynucleotides encoding galactose permeases and glucokinases.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to application PCT/US03/31544, filed Oct. 3, 2003, U.S. Provisional Application 60/416,166 filed Oct. 4, 2002 and U.S. Provisional Application 60/374,931 filed Oct. 4, 2002, which are hereby incorporated by reference.

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Summary of Invention Paragraph - BSTX (6):

[0005] In addition to the Embden-Meyerhof pathway, many bacteria posses an active transport system known as the phosphoenolpyruvate (PEP)-dependent phosphotransferase transport system (PTS). This system couples the transport of a carbon source, such as glucose to its phosphorylation. The phosphoryl group is transferred sequentially from PEP to enzyme I and from enzyme I to protein HPr. The actual translocation step is catalyzed by a family of membrane bound enzymes (called enzyme II), each of which is specific for one or a few carbon sources. Reference is made to Postma et al., (1993) Phosphoenolpyruvate: Carbohydrate Phosphotransferase Systems in Bacteria, Microbiol. Reviews. 57:543-594 and Postma P. W. (1996) Phosphotransferase System for Glucose and Other Sugars. In: Neidhardt et al., Eds. ESCHERICHIA

COLI AND SALMONELLA TYPHIMURIUM: CELLULAR AND MOLECULAR BIOLOGY. Vol.1. Washington, D.C. ASM Press pp 127-141. However, due to the fact that PTS metabolizes PEP to phosphorylate the carbon source, the PTS system decreases the efficiency of carbon substrate conversion to a desired product. In glycolysis, two molecules of PEP are formed for every molecule of glucose catabolized. However, one molecule of PEP is required for PTS to function, leaving only one molecule of PEP available for other biosynthetic reactions.

Summary of Invention Paragraph - BSTX (7):
[0006] Due to the role of PEP as a central metabolite, numerous approaches have been utilized to increase PEP supply in the cell and some of these are listed below:

Summary of Invention Paragraph - BSTX (16):
[0015] Contrary to the methods previously described, the present invention increases carbon flow to metabolic pathways in bacterial strains capable of transporting glucose without consuming PEP during the process. The conserved PEP or PEP precursors can then be redirected into a given metabolic pathway for enhanced production of a desired product. These strains are generated in cells having an inactivated PEP-dependent PTS by modifying an endogenous chromosomal regulatory region that is operably linked to a glucose assimilation protein and more specifically to a glucose transporter and/or a glucose phosphorylating protein, to restore or re-attain the ability of the cell to use glucose as a carbon source while maintaining an inactivated PTS. These cells are designated PTS.sup.-/Glu.sup.+.

Summary of Invention Paragraph - BSTX (18):
[0016] Accordingly, there is provided by the present invention a method for increasing carbon flow into a metabolic pathway of a bacterial host cell wherein the host cell was originally capable of utilizing a PTS for carbohydrate transport. The method comprises selecting a bacterial host cell which is phenotypically PTS.sup.-/Glu.sup.- and modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a polypeptide involved in glucose assimilation to restore the Glu.sup.+ phenotype.

Summary of Invention Paragraph - BSTX (19):
[0017] In a first aspect, the invention pertains to a method of increasing carbon flow into a metabolic pathway of a PTS.sup.-/Glu.sup.- bacterial host cell which was originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport which comprises a) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a glucose assimilation protein in a PTS.sup.-/Glu.sup.- host cell by transforming the PTS.sup.-/Glu.sup.- host cell with a DNA construct comprising a promoter and DNA flanking sequences corresponding to upstream (5') regions of the glucose assimilation protein; b) allowing integration of the DNA construct to restore a Glu+ phenotype; and c) culturing the transformed host cell under suitable culture conditions, wherein the carbon flow into a metabolic pathway of the transformed host cell is increased compared to the carbon flow into the same metabolic pathway in a corresponding PTS bacterial host cell cultured under essentially the same culture conditions. In one embodiment of the method the promoter is a non-host cell promoter or a modified endogenous promoter. In a second embodiment the glucose assimilation protein is a glucose transporter, preferably a galactose permease obtained from E. coli or a glucose transporter having at least 80% sequence identity thereto. In a third embodiment the glucose assimilation protein is a phosphorylating protein, preferably a glucokinase obtained from E. coli or a glucokinase having at least 80% sequence identity thereto. In a fourth embodiment of the method the bacterial host cell is selected from the group consisting of E. coli cells,

Bacillus cells and Pantoea cells. In a fifth embodiment, the PTS.sup.-/Glu.sup.- host cell is obtained from a PTS cell by deletion of one or more genes selected from the group consisting of ptsI, ptsH and crr. In a sixth embodiment, the PTS.sup.-/Glu.sup.+ host cell is transformed with a polynucleotide encoding a protein selected from the group consisting of a transketolase, a transaldolase, a phosphoenolpyruvate synthase, DAHP synthase, DHQ synthase, DHQ dehydratase, shikimate dehydrogenase, shikimate kinase EPSP synthase and chorismate synthase.

Summary of Invention Paragraph - BSTX (22):

[0020] In a fourth aspect, the invention pertains to a method of **increasing carbon flow** into a metabolic pathway of a PTS.sup.-/Glu.sup.- bacterial host cell originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport which comprises a) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a galactose permease in a PTS.sup.-/Glu.sup.- host cell by transforming the PTS.sup.-/Glu.sup.- host cell with a first DNA construct comprising a promoter and DNA flanking sequences corresponding to upstream (5') regions of the galactose permease; b) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a glucokinase in the PTS.sup.-/Glu.sup.- host cell by transforming the PTS.sup.-/Glu.sup.- host cell with a second DNA construct comprising a promoter and DNA flanking sequences corresponding to upstream (5') regions of the glucokinase; c) allowing integration of the first and the second DNA constructs, wherein the first DNA construct replaces an endogenous promoter of the nucleic acid encoding the galactose permease and the second DNA construct replaces an endogenous promoter of the nucleic acid encoding the glucokinase wherein both the galactose permease and the glucokinase are expressed in the host cell and wherein said expression results in an **increase in carbon flow** into a metabolic pathway of the transformed host cell compared to carbon flow into the same metabolic pathway in the corresponding unaltered PTS.sup.-/Glu.sup.- bacterial cell. In one embodiment the metabolic pathway is the common aromatic pathway. In a second embodiment the method further comprises transforming the PTS.sup.-/Glu.sup.- host cell with a polynucleotide encoding a protein selected from the group consisting of a transketolase, a transaldolase and a phosphoenolpyruvate synthase.

Summary of Invention Paragraph - BSTX (24):

[0022] In a sixth aspect the invention pertains to a method of increasing **phosphoenolpyruvate (PEP) availability** in a bacterial host cell which comprises a) selecting a bacterial host cell having a PTS.sup.-/Glu.sup.- phenotype, wherein the bacterial host was originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport; b) modifying an endogenous chromosomal regulatory sequence of the selected bacterial host cell comprising transforming said selected bacterial host cell with a DNA construct comprising a promoter, wherein said DNA construct is chromosomally integrated into the selected bacterial host cell replacing an endogenous promoter which is operably linked to a nucleic acid encoding a glucose assimilation protein; c) culturing the transformed bacterial host cell under suitable conditions; and d) allowing expression of the glucose assimilation protein to obtain an altered host cell having a PTS.sup.-/Glu.sup.+ phenotype, wherein the **PEP availability is increased compared to the PEP availability** in a corresponding unaltered PTS bacterial host cell cultured under essentially the same culture conditions. In one embodiment the glucose assimilation protein is a galactose permease and the DNA construct comprises an exogenous promoter which replaces the endogenous promoter of the galactose permease. In another embodiment the glucose assimilation protein is a glucokinase and the DNA construct comprises an exogenous promoter which replaces the endogenous promoter of a glucokinase.

Brief Description of Drawings Paragraph - DRTX (3):

[0027] The following abbreviations are used in the figure and throughout the disclosure: PEP=phosphoenolpyruvate; DAHP=3-deoxy-D-arabino-heptuloso- nate 7-phosphate; DHQ=3-dehydroquinate; DHS=3-dehydroshikimate; SHK=shikimate; S3P=shikimate 3-phosphate; EPSP=5-enolpyruvyl shikimate 3-phosphate; PHE=phenylalanine; TYR=tyrosine; TRP=tryptophan; Pyk=pyruvate kinase, which is encoded by the gene pyk; and Ppc=PEP carboxylase, which is encoded by the gene ppc. Further, the following genes are illustrated for the common aromatic pathway: aroB which encodes DHQ synthase; aroD which encodes DHQ dehydratase; aroE which encodes shikimate dehydrogenase; aroL and aroK which encode shikimate kinase; aroA which encodes EPSP synthase and aroC which encodes chorismate synthase. While not specifically illustrated, one skilled in the art is aware that aroG, aroF and aroH encode the three isozymes of DAHP synthase which catalyzes the conversion of Erythrose-4P (E4P) and PEP to DAHP in E. coli. FIG. 1B illustrates the varied compounds, the production of which, may be enhanced by an **increase in carbon flux and PEP availability** according to the methods encompassed by the invention.

Detail Description Paragraph - DETX (12):

[0051] "Increased **phosphoenolpyruvate (PEP) availability" means increasing the amount of intracellular PEP** which enhances carbon committed to a metabolic or productive pathway, said PEP which would otherwise have been metabolized in the PTS for phosphorylation of glucose.

Detail Description Paragraph - DETX (13):

[0052] The phrase "**increasing carbon flow" means increasing** the availability of carbon substrates to metabolic or productive pathways, said carbon substrate which would otherwise be diverted by the metabolism of PEP in the PTS. Carbon flow to a particular pathway can be measured by well know methods such as gas chromatography and mass spectroscopy. Carbon flow as measured by produced product may be at least 2%, 5%, 10%, 15%, 20%, 25%, 30%, or greater than the carbon flow in a corresponding PTS cell grown under essentially the same growth conditions.

Detail Description Paragraph - DETX (62):

[0100] The present invention is directed to a method for **increasing carbon flow** into a desired metabolic pathway of a host cell originally capable of utilizing a PTS for carbohydrate transport, said method including the steps of selecting a host cell which is effectively phenotypically PTS.sup.- and modifying at least one homologous chromosomal regulatory region, which is operably linked to a chromosomal nucleic acid which encodes a polypeptide involved in glucose assimilation, resulting in the restoration of a glucose.sup.+ phenotype and thereby **increasing the carbon flow** into and through a desired metabolic pathway.

Detail Description Paragraph - DETX (64):

[0102] A general review of the PTS can be found in (Postma et al., 1993, Microbiol. Rev. 57:543-594; Romano et al., 1979, J. Bacteriol. 139:93-97 and Saier et al. 1990, In: BACTERIAL ENERGETICS pp. 273-299, T. A. Krulwich, Ed. Academic Press, NY). Host cells or strains useful in the present invention include any organism capable of utilizing a PTS system for carbohydrate transport. This includes prokaryotes belonging to the genus Escherichia, Corynebacterium, Brevibacterium, Bacillus, Pseudomonas, Streptomyces, Pantoea or Staphylococcus. A list of suitable organisms is provided in Table 1. The inactivation of the PTS in any of these organisms should potentially **increase carbon flux and PEP (and PEP precursor) availability** in the cell for alternative metabolic routes and consequently could increase production of

desired compounds (e.g., aromatics) from such cells.

Detail Description Paragraph - DETX (75):
[0113] However, to increase or redirect carbon flow to desired metabolic pathways in inactivated PTS host cells, glucose transport and phosphorylation must be deregulated or amplified.

Detail Description Paragraph - DETX (124):
[0162] It should be noted that as the host cell is cultured in conditions which create an increase in carbon flow into the aromatic pathway, it may be necessary to identify and overcome rate-limiting steps in the pathway. This methodology is available to the artisan, see, for example, U.S. Pat. Nos. 5,168,056 and 5,776,736.

Detail Description Paragraph - DETX (127):
[0165] In addition to increasing the carbon flux through the aromatic pathway, the following genes may be overexpressed in PTS.sup.-/Glu.sup.+ cells according to the invention: pps which encodes PEP synthase in E. coli (see U.S. Pat. No. 5,985,617) and talA which encodes transaldolase (Iida et al. (1993) J. Bacterial. 175:5375-5383). Further any gene encoding an enzyme that catalyzes reactions within the common aromatic pathway (for example, DAHP synthase (aroF, aroG, aroH), DHQ synthase (aroB), DHQ dehydratase (aroD), shikimate dehydrogenase (aroE), shikimate kinase (aroL, aroK), EPSP synthase (aroA) and chorismate synthase (aroC) may be amplified in the PTS.sup.-/Glu.sup.+ cells encompassed by the present invention.

Detail Description Paragraph - DETX (135):
[0173] Thus, having provided a PTS.sup.-/Glu.sup.+ strain which conserves PEP resulting in an increase in carbon flux into a metabolic pathway, such as the aromatic amino acid pathway, glycolysis, the TCA cycle, and the pentose phosphate pathway, by redirecting PEP and PEP precursors, the inventors have provided a host system which can be utilized for enhanced production of desired compounds in comparison to the production of the same compounds in a corresponding PTS host cell.

Detail Description Paragraph - DETX (187):
[0220] By constitutively expressing galP on the chromosome from the trc promoter the flux of carbon from glucose was increased into the pathway for the desired products, glycerol and 1,3-propanediol rather than into pathways to produce cell mass.

Claims Text - CLTX (1):
1. A method of increasing carbon flow into a metabolic pathway of a PTS.sup.-/Glu.sup.- bacterial host cell which was originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport comprising, a) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a glucose assimilation protein in a PTS.sup.-/Glu.sup.- host cell by transforming the PTS.sup.-/Glu.sup.- host cell with a DNA construct comprising a promoter and DNA flanking sequences corresponding to upstream (5') regions of the glucose assimilation protein; b) allowing integration of the DNA construct to restore a Glu+ phenotype; and c) culturing the transformed host cell under suitable culture conditions, wherein the carbon flow into a metabolic pathway of the transformed host cell is increased compared to the carbon flow into the same metabolic pathway in a corresponding PTS bacterial host cell cultured under essentially the same culture conditions.

Claims Text - CLTX (22):
22. A method of increasing carbon flow into a metabolic pathway of a

PTS.sup.-/Glu.sup.- bacterial host cell originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport comprising, a) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a galactose permease in a PTS.sup.-/Glu.sup.- host cell by transforming the PTS.sup.-/Glu.sup.- host cell with a first DNA construct comprising a promoter and DNA flanking sequences corresponding to upstream (5') regions of the galactose permease; b) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a glucokinase in the PTS.sup.-/Glu.sup.- host cell by transforming the PTS.sup.-/Glu.sup.- host cell with a second DNA construct comprising a promoter and DNA flanking sequences corresponding to upstream (5') regions of the glucokinase; c) allowing integration of the first and the second DNA constructs, wherein the first DNA construct replaces an endogenous promoter of the nucleic acid encoding the galactose permease and the second DNA construct replaces an endogenous promoter of the nucleic acid encoding the glucokinase wherein both the galactose permease and the glucokinase are expressed in the host cell and wherein said expression results in an increase in carbon flow into a metabolic pathway of the transformed host cell compared to carbon flow into the same metabolic pathway in a corresponding unaltered PTS.sup.-/Glu.sup.- bacterial cell.

Claims Text - CLTX (34):

34. A method of increasing phosphoenolpyruvate (PEP) availability in a bacterial host cell comprising, a) selecting a bacterial host cell having a PTS.sup.-/Glu.sup.- phenotype, wherein the bacterial host was originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport; b) modifying an endogenous chromosomal regulatory sequence of the selected bacterial host cell comprising transforming said selected bacterial host cell with a DNA construct comprising a promoter, wherein said DNA construct is chromosomally integrated into the selected bacterial host cell replacing an endogenous promoter which is operably linked to a nucleic acid encoding a glucose assimilation protein; c) culturing the transformed bacterial host cell under suitable conditions; and d) allowing expression of the glucose assimilation protein to obtain an altered host cell having a PTS.sup.-/Glu.sup.+ phenotype, wherein the PEP availability is increased compared to the PEP availability in a corresponding unaltered PTS bacterial host cell cultured under essentially the same culture conditions.

PGPUB-DOCUMENT-NUMBER: 20040019931

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040019931 A1

TITLE: Methods and compositions for modifying oil and protein content in plants

PUBLICATION-DATE: January 29, 2004

INVENTOR-INFORMATION:

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APPL-NO: 09/ 930906

DATE FILED: August 16, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60226142 20000818 US

US-CL-CURRENT: 800/281, 435/194 , 435/320.1 , 435/419 , 435/69.1 , 536/23.2

ABSTRACT:

Compositions and methods for altering pyruvate synthesis in plants are provided. The compositions and methods find use in increasing the oil or protein content of plant tissues, particularly seeds. Novel isolated nucleotide molecules encoding cytosolic and plastidic pyruvate kinases are provided. The methods involve introducing into a plant nucleotide sequences encoding enzymes that alter pyruvate synthesis. Additionally provided are isolated pyruvate kinases and transformed plants, plant tissues, plant cells, and seeds thereof.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 60/226,142, filed Aug. 18, 2000, which is hereby incorporated herein in its entirety by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (24):

[0021] If desired, one or more additional nucleotide constructs may be introduced into the plant. Such additional nucleotide constructs can comprise, for example, at least a portion of a nucleotide sequence of an enzyme, or other protein, that is capable of affecting the synthesis of pyruvate in the cytosol. If expression of the nucleotide sequence is desired, the nucleotide construct can additionally comprise an operably linked promoter. The invention does not depend on a particular nucleotide sequence. Any nucleotide sequence originating from a gene or transcript that encodes an enzyme or protein that is capable of increasing or decreasing the synthesis and/or level of pyruvate in the cytosol can be employed. Typically, such an enzyme or protein is capable of altering the amount of **PEP that is available** in the cytosol for the

formation of pyruvate. If a decreased level or activity of such an enzyme or protein is desired, the methods of the invention may additionally comprise anti-sense suppression, co-suppression, or chimeraplasty.

Summary of Invention Paragraph - BSTX (29):
[0026] Methods are provided for altering the metabolism of pyruvate in plastids. The methods find use in increasing the synthesis of fatty acids and/or oil in a plant, or part thereof. In particular, the methods find use in the production of improved cultivars of crop plants having seeds with increased levels of oil. By increasing the flux of carbon toward oil biosynthesis, increased levels of oil, fatty acids, oil precursors, and specialty molecules that are synthesized from oil precursors can be achieved in a plant, or part thereof. The methods involve introducing into a plant at least one nucleotide construct comprising at least a portion of a nucleotide sequence encoding a pyruvate kinase or an NADP.sup.+-dependent malic enzyme. In some embodiments of the invention, both a pyruvate kinase nucleotide sequence and a NADP.sup.+-dependent malic enzyme nucleotide sequence are introduced into the plant. Such nucleotide sequences can be introduced into the plant as a single nucleotide molecule comprising both the pyruvate kinase and a NADP.sup.+-dependent malic enzyme nucleotide sequences. Alternatively, the pyruvate kinase and a NADP.sup.+-dependent malic enzyme nucleotide sequences can be introduced into the plant on two separate nucleotide molecules.

Summary of Invention Paragraph - BSTX (33):
[0030] If desired, one or more additional nucleotide constructs may be introduced into the plant. Such additional nucleotide constructs can comprise, for example, at least a portion of a nucleotide sequence encoding an enzyme, or other protein, that is capable of affecting the synthesis or the level of pyruvate in the plastid. If expression of the nucleotide sequence is desired, the nucleotide construct can additionally comprise an operably linked promoter. The invention does not depend on a particular nucleotide sequence. Any nucleotide sequence that encodes an enzyme, or protein, that is capable of increasing or decreasing the synthesis of pyruvate in the cytosol can be employed. Typically, such an enzyme or protein is capable of altering the amount of PEP, malate, or both that is available for the formation of pyruvate within the plastid. Such an enzyme can be localized in any compartment or organelle of the cell, including, but not limited to, the cytosol, plastids, mitochondria, and vacuoles. If a decreased level or activity of such an enzyme or protein is desired, the methods of the invention may additionally comprise anti-sense suppression, co-suppression, chimeraplasty, and dominant-negative strategies.

Summary of Invention Paragraph - BSTX (38):
[0035] To reduce or eliminate the conversion of malate to pyruvate in mitochondria, the plant can be transformed with a nucleotide construct comprising at least a portion of an NAD.sup.+-dependent malic enzyme nucleotide sequence. Malate that is formed in the cytosol, instead of entering the choloroplast, can enter the mitochondria where it can be decarboxylated to form pryuvate, which can then be converted into acetyl-CoA. Acetyl-CoA can then enter the TCA cycle. Reducing or eliminating the decarboxylation of malate in plant mitochondria by antisense suppression of mitochondrial NAD.sup.+-dependent malic enzyme is known to increase the flux of carbon to plastids in plant storage tissues. See WO 98/23757, herein incorporated by reference.

Summary of Invention Paragraph - BSTX (40):
[0037] While the methods of the present invention do not depend on a particular biological mechanism for increasing the oil or other desired product, it is recognized the methods of the present invention can lead to a

disruption in the metabolism of the plant leading to an increased flux of carbon flux to the plastids. This increased carbon flux may be the result of an increased rate of transport of malate from the cytosol to the plastids. Other cytosolic metabolites may, however, contribute to the increased flux of carbon to the plastid. Such metabolites include, but are not limited to, 3-phosphoglyceric acid and dihydroxyacetone phosphate.

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	5111	carbon near2 (flux or flow)	US-PGPUB; USPAT	OR	OFF	2005/05/13 15:33
L2	374	1 near4 (modif$8 or alter$8 or increas$8)	US-PGPUB; USPAT	OR	OFF	2005/05/13 15:33
L3	233	(phosphoenol adj pyruvate or pep or phosphoenolpyruvate or phospho adj enol adj pyruvate) near4 (suppl$4 or availab$8)	US-PGPUB; USPAT	OR	OFF	2005/05/13 15:34
L4	11	2 and 3	US-PGPUB; USPAT	OR	OFF	2005/05/13 15:34
L5	8702	phosphotransferase$1 or phospho adj transferase$1	US-PGPUB; USPAT	OR	OFF	2005/05/13 15:51
L6	58	(2 or 3) and 5	US-PGPUB; USPAT	ADJ	OFF	2005/05/13 15:51

PGPUB-DOCUMENT-NUMBER: 20050102716

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20050102716 A1

TITLE: Transgenic plants containing altered levels of sterol compounds and tocopherols

PUBLICATION-DATE: May 12, 2005

INVENTOR-INFORMATION:

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APPL-NO: 10/ 647517

DATE FILED: January 16, 2004

RELATED-US-APPL-DATA:

child 10647517 A1 20040116

parent continuation-of 09548256 20000412 US ABANDONED

non-provisional-of-provisional 60128995 19990412 US

US-CL-CURRENT: 800/287, 435/468

ABSTRACT:

Provided are recombinant constructs comprising DNA sequences encoding enzymes effective in altering the biosynthesis and accumulation of sterol compounds and tocopherols in transgenic plants. Also provided are methods of using such constructs to produce transgenic plants, seeds of which contain elevated levels of sitostanol and/or sitostanol esters, and .alpha.-tocopherol, as well as reduced levels of campesterol and campestanol and their corresponding esters. These seeds also contain the novel sterol brassicastanol. Oil obtained from seeds of such transgenic plants is also provided. This oil can be used to prepare food and pharmaceutical compositions effective in lowering the level of low density lipoprotein cholesterol in blood serum. In addition, novel DNA sequences encoding plant steroid 5.alpha.-reductases are also disclosed.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of Ser. No. 09/548,256, filed Apr. 12, 2000 and claims priority from provisional application 60/128,995, filed Apr. 12, 1999, which is hereby incorporated by reference in its entirety for all purposes.

---------- KWIC --------

Detail Description Paragraph - DETX (252):

[0360] All of the enzymes discussed herein can be modified for plastid targeting by employing plant cell nuclear transformation constructs wherein DNA coding sequences of interest are fused to any of the available transit peptide sequences capable of facilitating transport of the encoded enzymes into plant plastids, and driving expression by employing an appropriate promoter such as any of those discussed above. Targeting of enzymes involved in altering sterol compound and tocopherol quantity and/or quality to plastids can be achieved by fusing DNA encoding plastid, e.g., chloroplast, leucoplast, amyloplast, etc., transit peptide sequences to the 5'-ATG of DNAs encoding enzymes affecting the biosynthesis and accumulation of these compounds. The sequences that encode a transit peptide region can be obtained, for example, from plant nuclear-encoded plastid proteins, such as the small subunit (SSU) of ribulose bisphosphate carboxylase, EPSP synthase, plant fatty acid biosynthesis related genes including fatty acyl-ACP thioesterases, acyl carrier protein (ACP), stearoyl-ACP desaturase, .beta.-ketoacyl-ACP synthase and acyl-ACP thioesterase, or LHCPII genes, etc. Plastid transit peptide sequences can also be obtained from nucleic acid sequences encoding carotenoid biosynthetic enzymes, such as GGPP synthase, phytoene synthase, and phytoene desaturase. Other transit peptide sequences useful in the present invention are disclosed in Von Heijne et al. (1991) Plant Mol. Biol. Rep. 9: 104; Clark et al. (1989) J. Biol. Chem. 264: 17544; della-Cioppaet al. (1987) Plant Physiol. 84: 965; Romer et al. (1993) Biochem. Biophys. Res. Commun. 196: 1414; and Shah et al. (1986) Science 233: 478. Plant sterol compound/tocopherol biosynthetic enzyme-encoding sequences useful in the present invention can utilize native or heterologous transit peptides. The encoding sequence for a transit peptide effective in transport to plastids can include all or a portion of the encoding sequence for a particular transit peptide, and may also contain portions of the mature protein encoding sequence associated with a particular transit peptide. Numerous examples of transit peptides that can be used to deliver target proteins into plastids exist, and the particular transit peptide encoding sequences useful in the present invention are not critical as long as delivery into a plastid is obtained. Proteolytic processing within the plastid then produces the mature enzyme. This technique has proven successful not only with enzymes involved in polyhydroxyalkanoate biosynthesis (Nawrath et al. (1994) Proc. Natl. Acad. Sci. USA 91: 12760), but also with neomycin phosphotransferase II (NPT-II) and CP4 EPSPS (Padgette et al. (1995) Crop Sci. 35: 1451), for example.

Detail Description Paragraph - DETX (296):

[0398] Total sterols increased by 3.2- and 3.9-fold in the best performing plants (transgenic events 3 and 4). These two events also showed the highest increases of individual sterols. Campesterol increased by 2.7-fold, sitosterol by 3.4-fold, sitostanol by 3.2-fold and other sterols by 6.5-fold in event 3 while stigmasterol increased by 2.3-fold in event 4. The other sterols, which account for the highest increase in total sterols, were pathway intermediates that included squalene, cycloartenol, 24-methylene cycloartenol, obtusifoliol, isofucosterol, and stigmasta-7-enol. These pathway intermediates normally form minor constituents in the sterol composition of seeds. However, in the transgenic seeds, probably due to increased carbon flux through the pathway, they accumulate in significant amounts. This suggests additional control points for sterol biosynthesis in plants such as squalene epoxidase, C-24 sterol methyltransferase, and C-14 obtusifoliol demethylase.

Detail Description Paragraph - DETX (306):

[0404] The decrease in the amount of campesterol is consistent with the expected activity of the SMTII enzyme. This enzyme catalyzes the reaction 18 in Scheme 1. The substrate for this reaction, which is 24-methylene lophenol, can also undergo reaction 18b which is a C-4 demethylation. This latter route leads to the formation of 24-niethyl sterols such as campesterol. It is presumed that increased activity of SMTII due to the higher expression of the introduced Arabidopsis thaliana SMTII gene allows for increased carbon flux through the pathway leading to sitosterol and thus reducing the availability of 24-methylene lophenol for reaction 18b which reduces the amount of campesterol formed.

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TITLE: Fermentative production of d-hydroxyphenylglycine and d-phenylglycine

PUBLICATION-DATE: April 28, 2005

INVENTOR-INFORMATION:

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US-CL-CURRENT: 435/108

ABSTRACT:

A new fermentative process for the preparation of D-p-hydroxyphenylglycine (D-HPG) or D-henylglycine (D-pG) in enantiomerically pure form is disclosed. Precursors for the formation of D-HPG and D-pG are withdrawn form the common aromatic amino acid pathway, converted to p-hydroxyphenylglyoxylate or phenylglyoxylate, and are finally converted to D-HPG or D-pG by the action of a stero-inverting D-aminotransferase.

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Summary of Invention Paragraph - BSTX (20):
[0019] Suitable measures to increase the availability of HPP and PP in said microorganisms comprise the selection of microorganisms showing a beneficial spontaneous mutation, the selection of microorganisms that evolve from classical strain improvement programs involving random mutagenesis, and the selection of microorganisms with improved production capabilities that evolved from application of recombinant DNA technology. Suitable measures to increase the availability of HPP and PP, e.g., can be the introduction of feedback

resistant mutants of key enzymes or the overexpression of one or several pathway enzymes as reviewed by Berry (1996, TIBTECH, 14: 250-256). Application of recombinant DNA technology can also be directed towards the introduction of phosphoenolpyruvate-inde- pendent sugar uptake systems (WO 98/18936), towards the deletion of the phosphotransferase system (PTS) (Berry, 1996, TIBTECH, 14: 250-256) and/or towards an increased availability of phosphoenolpyruvate by modifications of reactions acting on the intracellular phosphoenolpyruvate concentration (Berry, 1996, TIBTECH, 14: 250-256). Application of recombinant DNA technology can also be directed towards introducing increased intracellular transketolase and/or transaldolase activity (WO 98/18936).

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DOCUMENT-IDENTIFIER: US 20050086713 A1

TITLE: Transgenic plants containing altered levels of steroid compounds

PUBLICATION-DATE: April 21, 2005

INVENTOR-INFORMATION:

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US-CL-CURRENT: 800/278, 435/189 , 435/419 , 435/468

ABSTRACT:

Disclosed are constructs comprising sequences encoding 3-hydroxy-3methylglutaryl-Coenzyme A reductase and at least one other sterol synthesis pathway enzyme. Also disclosed are methods for using such constructs to alter sterol production and content in cells, plants, seeds and storage organs of plants. Also provided are oils and compositions containing altered sterol levels produced by use of the disclosed constructs. Novel nucleotide sequences useful in the alteration of sterol production are also provided. Also provided are cells, plants, seeds and storage organs of plants comprising sequences encoding 3-hydroxy-3methylglutaryl-Coenzyme A reductase, at least one other sterol synthesis pathway enzyme and at least one tocopherol synthesis enzyme.

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Brief Description of Drawings Paragraph - DRTX (8):

[0052] FIG. 6 is a map showing the structure of construct pMON43818. pMON43818 is a recombinant binary vector carrying the gene encoding rubber hydroxymethyl glutaryl CoA reductasel (HMGR1) in sense orientation driven by

the soybean alpha' beta conglycinin promoter. P-NOS: nopaline synthase gene promoter; kan: coding region for neomycin phospho transferase protein to confer resistance to kanamycin; NOS 3': 3' termination end of nopaline synthase coding region; Soy Alpha' Beta Conglycinin: 7S alpha' beta conglycinin gene promoter from soybean; Rubber HMGR1 gene: coding sequence for HMGR1 gene from Hevea brasiliensis; E9 3': 3' end of pea rbcS E9 gene; Left border: octopine left border, sequence essential for transfer of T-DNA into Agrobacterium; ori-V: plasmid origin of replication in Agrobacterium; rop: coding sequence for repressor of primer; Ori-322: origin of replication in E.coli; Spc/Str: coding region for Tn7 adenylyltransferase (AAD(3")) conferring resistance to spectinomycin and streptomycin; Right Border: right border sequence of T-DNA essential for integration into Agrobacterium.

Brief Description of Drawings Paragraph - DRTX (9):

[0053] FIG. 7 is a map showing the structure of construct pMON43052. pMON43052 is a recombinant shuttle vector, carrying the cDNA fragment encoding the catalytic domain of Arabidopsis HMGR1 in sense orientation driven by the soybean alpha' beta conglycinin promoter. P-NOS: nopaline synthase gene promoter; kan: coding region for neomycin phosphotransferase protein to confer resistance to kanamycin; NOS 3': 3' termination end of nopaline synthase coding region; Soy Alpha' Beta Conglycinin: 7S alpha' beta conglycinin gene promoter from soybean; Arabidopsis HMGR catalytic domain: coding sequence for the catalytic domain of Arabidopsis HMGR1 protein; E9 3': 3' end of pea rbcs E9 gene; Left border: octopine left border, sequence essential for transfer of T-DNA into Agrobacterium; ori-V: plasmid origin of replication in Agrobacterium; rop: coding sequence for repressor of primer; Ori-322: origin of replication in E.coli; Spc/Str: coding region for Tn7 adenylyltransferase (AAD(3")) conferring resistance to spectinomycin and streptomycin; Right Border: right border sequence of T-DNA essential for integration into Agrobacterium.

Brief Description of Drawings Paragraph - DRTX (10):

[0054] FIG. 8 is a map showing the structure of construct pMON51850. pMON51850 is a binary vector for Agrobacterium mediated transformation of soybean. P-NOS: nopaline synthase gene promoter; kan: coding region for neomycin phosphotransferase protein to confer resistance to kanamycin; NOS 3': 3' termination end of nopaline synthase coding region; Left border: octopine left border sequence essential for transfer of T-DNA into Agrobacterium; ori-V: plasmid origin of replication in Agrobacterium; rop: coding sequence for repressor of primer; ori-322: origin of replication in E.coli; Spc/Str: coding region for Tn7 adenylyltransferase (AAD(3")) conferring resistance to spectinomycin and streptomycin; Right Border: right border sequence of T-DNA essential for integration into Agrobacterium.

Brief Description of Drawings Paragraph - DRTX (11):

[0055] FIG. 9 is a map showing the structure of construct pMON43057. pMON43057 is a recombinant binary vector for Agrobacterium mediated transformation of soybean, carrying the gene cassette for expressing catalytic domain of HMGR1 from Arabidopsis thaliana. The catalytic domain of the HMGR1 cDNA is driven by soybean 7S alpha' beta conglycinin promoter. P-NOS: nopaline synthase gene promoter; kan: coding region for neomycin phosphotransferase protein to confer resistance to kanamycin; NOS 3': 3' termination end of nopaline synthase coding region; Left border: octopine left border sequence essential for transfer of T-DNA into Agrobacterium; ori-V: plasmid origin of replication in Agrobacterium; rop: coding sequence for repressor of primer; ori-322: origin of replication in E.coli; Spc/Str: coding region for Tn7

adenylyltransferase (AAD(3")) conferring resistance to spectinomycin and streptomycin; Right Border: right border sequence essential for transfer of T-DNA into Agrobacterium; Soy Alpha' Beta Conglycinin: soybean 7S alpha' beta conglycinin gene promoter; Arabidopsis HMGR catalytic domain: coding sequence for Arabidopsis HMGR1 catalytic domain; E9 3': 3' end of pea rbcS E9 gene.

Brief Description of Drawings Paragraph - DRTX (12):

[0056] FIG. 10 is a map showing the structure of construct pMON43058. pMON43058 is a recombinant binary vector for Agrobacterium-mediated soybean transformation, carrying gene expression cassettes for catalytic domain of HMGR1 from Arabidopsis thaliana and SMTII from Arabidopsis thaliana. P-NOS: nopaline synthase gene promoter; kan: coding region for neomycin phosphotransferase protein to confer resistance to kanamycin; NOS 3': 3' termination end of nopaline synthase coding region; Left border: octopine left border sequence essential for transfer of T-DNA into Agrobacterium; ori-V: plasmid origin of replication in Agrobacterium; rop: coding sequence for repressor of primer; ori-322: origin of replication in E.coli; Spc/Str: coding region for Tn7 adenylyltransferase (AAD(3")) conferring resistance to spectinomycin and streptomycin; Right Border: right border sequence essential for transfer of T-DNA into Agrobacterium; Soy Alpha' Beta Conglycinin: 7S alpha' beta conglycinin gene promoter from soybean; Arabidopsis HMGR catalytic domain: sequence encoding the catalytic domain of Arabidopsis HMGR1; E9 3': 3' end of pea rbcS E9 gene; Soy Alpha' Beta Conglycinin: soybean 7S alpha'beta conglycinin gene promoter; Arabidopsis SMT2: cDNA encoding sterol methyl transferase II enzyme from Arabidopsis thaliana (accession no: X89867); NOS 3': 3' termination end of nopaline synthase coding region.

Detail Description Paragraph - DETX (208):

[0285] In preferred embodiments, the vector used to express the polypeptide coding gene includes a selection marker that is effective in a plant cell, preferably a drug resistance selection marker. One preferred drug resistance marker is the gene whose expression results in kanamycin resistance; i.e., the chimeric gene containing the nopaline synthase promoter, Tn5 neomycin phosphotransferase II and nopaline synthase 3' nontranslated region described by Rogers et al., in Methods for Plant Molecular Biology, A. Weissbach and H. Weissbach eds., Academic Press Inc., San Diego, Calif. (1988).

Detail Description Paragraph - DETX (344):

[0419] Total sterols increased by 3.2- and 3.9-fold in the best performing plants (transgenic events 3 and 4). These two events also showed the highest increases of individual sterols. Campesterol increased by 2.7-fold, sitosterol by 3.4-fold, sitostanol by 3.2-fold and other sterols by 6.5-fold in event 3 while stigmasterol increased by 2.3-fold in event 4. The other sterols, which account for the highest increase in total sterols were pathway intermediates that included squalene, cycloartenol, 24-methylene cycloartenol, obtusifoliol, isofucosterol, and stigmasta-7-enol. These pathway intermediates normally form minor constituents in the sterol composition of seeds. However, in the transgenic seeds, probably due to increased carbon flux through the pathway, they accumulate in significant amounts. This suggests additional control points for sterol biosynthesis in plants such as squalene epoxidase, C-24 sterol methyltransferase, and C-14 obtusifoliol demethylase.

Detail Description Paragraph - DETX (356):

[0430] Six transgenic lines haboring pMON43058 produced 5.8- to 6-fold increase in total sterols and the rest of the 10 transgenic lines with the pMON43058 showed 3- to 5-fold increase in total sterols. The best performing transgenic lines showed about 2- to 3-fold increase in sitosterol and 4.5- to 6-fold increase in sitostanol levels. However, the campesterol accumulation

was reduced by 50% in these lines. This was due to overexpression of the Arabidopsis SMTII enzyme which enhances the carbon flux towards the synthesis of 24-ethyl sterols thereby reducing the carbon flux through the pathway leading to the synthesis of 24-methyl sterols. As seen in pMON43057 transgenic lines, all of the transgenic lines harboring the pMON43058 also accumulated 50-60% of the total sterols in the form of pathway intermediates which are squalene, cycloartenol, 24-methylene cycloartenol, obtusifoliol, isofucosterol, and stigmasta-7-enol. These pathway intermediates normally form minor constituents in the sterol composition of seeds. However, in the transgenic seeds, probably due to increased carbon flux through the pathway, they accumulate in significant amounts. The pathway intermediates accumulation is highly significant when the truncated from of HMGR is overexpressed as compared to the full length form of HMGR suggesting that the overexpression of the truncated form of HMGR creates even greater increase in carbon flux through the pathway. This provides further evidence for additional control points for sterol biosynthesis in plants such as squalene epoxidase, sterol methyltransferase I, sterol C4-demethylase, obtusifoliol C14.alpha.-demethylase, sterol C5-desaturase, and sterol methyl transferase II.

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TITLE: Glucose transport mutants for production of biomaterial

PUBLICATION-DATE: April 14, 2005

INVENTOR-INFORMATION:

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COUNTRY	APPL-NO	DOC-ID	APPL-DATE
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US-CL-CURRENT: 435/471

ABSTRACT:

A method is disclosed for restoring a Glu.sup.+ phenotype to a PTS.sup.-/Glu.sup.- bacterial cell which was originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport. Bacterial cells comprising the Glu.sup.+ phenotype have modified endogenous chromosomal regulatory regions which are operably linked to polynucleotides encoding galactose permeases and glucokinases.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to application PCT/US03/31544, filed Oct. 3, 2003, U.S. Provisional Application 60/416,166 filed Oct. 4, 2002 and U.S. Provisional Application 60/374,931 filed Oct. 4, 2002, which are hereby incorporated by reference.

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Abstract Paragraph - ABTX (1):

A method is disclosed for restoring a Glu.sup.+ phenotype to a PTS.sup.-/Glu.sup.- bacterial cell which was originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport. Bacterial cells comprising the Glu.sup.+ phenotype have modified endogenous chromosomal regulatory regions which are operably linked to polynucleotides encoding galactose permeases and glucokinases.

Summary of Invention Paragraph - BSTX (2):

[0002] The present invention relates to genetically engineering metabolic pathways in bacterial host cells and provides methods and systems for the production of desired products in the engineered host cells. In particular,

the invention relates to the enhancement of glucose transport in host strains, which were originally capable of using a phosphoenolpyruvate (PEP):phosphotransferase transport system (PTS) for glucose transport, by reducing PTS phosphoenolpyruvate (PEP) consumption and redirecting PEP or PEP precursors into a desired metabolic pathway, such as the common aromatic amino acid pathway.

Summary of Invention Paragraph - BSTX (6):
[0005] In addition to the Embden-Meyerhof pathway, many bacteria posses an active transport system known as the phosphoenolpyruvate (PEP)-dependent phosphotransferase transport system (PTS). This system couples the transport of a carbon source, such as glucose to its phosphorylation. The phosphoryl group is transferred sequentially from PEP to enzyme I and from enzyme I to protein HPr. The actual translocation step is catalyzed by a family of membrane bound enzymes (called enzyme II), each of which is specific for one or a few carbon sources. Reference is made to Postma et al., (1993) Phosphoenolpyruvate: Carbohydrate Phosphotransferase Systems in Bacteria, Microbiol. Reviews. 57:543-594 and Postma P. W. (1996) Phosphotransferase System for Glucose and Other Sugars. In: Neidhardt et al., Eds. ESCHERICHIA COLI AND SALMONELLA TYPHIMURIUM: CELLULAR AND MOLECULAR BIOLOGY. Vol.1. Washington, D.C. ASM Press pp 127-141. However, due to the fact that PTS metabolizes PEP to phosphorylate the carbon source, the PTS system decreases the efficiency of carbon substrate conversion to a desired product. In glycolysis, two molecules of PEP are formed for every molecule of glucose catabolized. However, one molecule of PEP is required for PTS to function, leaving only one molecule of PEP available for other biosynthetic reactions.

Summary of Invention Paragraph - BSTX (7):
[0006] Due to the role of PEP as a central metabolite, numerous approaches have been utilized to increase PEP supply in the cell and some of these are listed below:

Summary of Invention Paragraph - BSTX (16):
[0015] Contrary to the methods previously described, the present invention increases carbon flow to metabolic pathways in bacterial strains capable of transporting glucose without consuming PEP during the process. The conserved PEP or PEP precursors can then be redirected into a given metabolic pathway for enhanced production of a desired product. These strains are generated in cells having an inactivated PEP-dependent PTS by modifying an endogenous chromosomal regulatory region that is operably linked to a glucose assimilation protein and more specifically to a glucose transporter and/or a glucose phosphorylating protein, to restore or re-attain the ability of the cell to use glucose as a carbon source while maintaining an inactivated PTS. These cells are designated PTS.sup.-/Glu.sup.+.

Summary of Invention Paragraph - BSTX (18):
[0016] Accordingly, there is provided by the present invention a method for increasing carbon flow into a metabolic pathway of a bacterial host cell wherein the host cell was originally capable of utilizing a PTS for carbohydrate transport. The method comprises selecting a bacterial host cell which is phenotypically PTS.sup.-/Glu.sup.- and modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a polypeptide involved in glucose assimilation to restore the Glu.sup.+ phenotype.

Summary of Invention Paragraph - BSTX (19):
[0017] In a first aspect, the invention pertains to a method of increasing carbon flow into a metabolic pathway of a PTS.sup.-/Glu.sup.- bacterial host cell which was originally capable of utilizing a phosphotransferase transport

system (PTS) for carbohydrate transport which comprises a) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a glucose assimilation protein in a PTS.sup.-/Glu.sup.- host cell by transforming the PTS.sup.-/Glu.sup.- host cell with a DNA construct comprising a promoter and DNA flanking sequences corresponding to upstream (5') regions of the glucose assimilation protein; b) allowing integration of the DNA construct to restore a Glu+ phenotype; and c) culturing the transformed host cell under suitable culture conditions, wherein the carbon flow into a metabolic pathway of the transformed host cell is **increased compared to the carbon flow** into the same metabolic pathway in a corresponding PTS bacterial host cell cultured under essentially the same culture conditions. In one embodiment of the method the promoter is a non-host cell promoter or a modified endogenous promoter. In a second embodiment the glucose assimilation protein is a glucose transporter, preferably a galactose permease obtained from E. coli or a glucose transporter having at least 80% sequence identity thereto. In a third embodiment the glucose assimilation protein is a phosphorylating protein, preferably a glucokinase obtained from E. coli or a glucokinase having at least 80% sequence identity thereto. In a fourth embodiment of the method the bacterial host cell is selected from the group consisting of E. coli cells, Bacillus cells and Pantoea cells. In a fifth embodiment, the PTS.sup.-/Glu.sup.- host cell is obtained from a PTS cell by deletion of one or more genes selected from the group consisting of ptsI, ptsH and crr. In a sixth embodiment, the PTS.sup.-/Glu.sup.+ host cell is transformed with a polynucleotide encoding a protein selected from the group consisting of a transketolase, a transaldolase, a phosphoenolpyruvate synthase, DAHP synthase, DHQ synthase, DHQ dehydratase, shikimate dehydrogenase, shikimate kinase EPSP synthase and chorismate synthase.

Summary of Invention Paragraph - BSTX (22):

[0020] In a fourth aspect, the invention pertains to a method of **increasing carbon flow** into a metabolic pathway of a PTS.sup.-/Glu.sup.- bacterial host cell originally capable of utilizing a **phosphotransferase** transport system (PTS) for carbohydrate transport which comprises a) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a galactose permease in a PTS.sup.-/Glu.sup.- host cell by transforming the PTS.sup.-/Glu.sup.- host cell with a first DNA construct comprising a promoter and DNA flanking sequences corresponding to upstream (5') regions of the galactose permease; b) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a glucokinase in the PTS.sup.-/Glu.sup.- host cell by transforming the PTS.sup.-/Glu.sup.- host cell with a second DNA construct comprising a promoter and DNA flanking sequences corresponding to upstream (5') regions of the glucokinase; c) allowing integration of the first and the second DNA constructs, wherein the first DNA construct replaces an endogenous promoter of the nucleic acid encoding the galactose permease and the second DNA construct replaces an endogenous promoter of the nucleic acid encoding the glucokinase wherein both the galactose permease and the glucokinase are expressed in the host cell and wherein said expression results in an **increase in carbon flow** into a metabolic pathway of the transformed host cell compared to carbon flow into the same metabolic pathway in the corresponding unaltered PTS.sup.-/Glu.sup.- bacterial cell. In one embodiment the metabolic pathway is the common aromatic pathway. In a second embodiment the method further comprises transforming the PTS.sup.-/Glu.sup.- host cell with a polynucleotide encoding a protein selected from the group consisting of a transketolase, a transaldolase and a phosphoenolpyruvate synthase.

Summary of Invention Paragraph - BSTX (23):

[0021] In a fifth aspect, the invention pertains to a method of restoring a Glu+phenotype to a PTS.sup.-/Glu.sup.- bacterial host cell which was originally

capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport which comprises a) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a glucose transporter in a PTS.sup.-/Glu.sup.- host cell by transforming the PTS.sup.-/Glu.sup.- host cell with a first DNA construct comprising a promoter and DNA flanking sequences corresponding to upstream (5') regions of the glucose transporter; b) allowing integration of the first DNA construct, wherein the first DNA construct replaces an endogenous promoter of the nucleic acid encoding the glucose transporter; and c) allowing expression of the glucose transporter, wherein said expression restores a Glu+ phenotype to the PTS.sup.-/Glu.sup.- host cell. In a preferred embodiment the method according to this aspect further comprises modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a glucokinase in the PTS.sup.-/Glu.sup.- host cell by transforming the PTS.sup.-/Glu.sup.- host cell with a second DNA construct comprising an exogenous promoter and DNA flanking sequences corresponding to upstream (5') regions of the glucokinase; allowing integration of the second DNA construct wherein the second DNA construct replaces an endogenous promoter of the nucleic acid encoding the glucokinase; and allowing expression of the glucokinase. In one embodiment the restored Glu.sup.+ cells have a specific growth rate of at least about 0.4 hr.sup.-1. In another embodiment the glucose transporter is a galactose permease.

Summary of Invention Paragraph - BSTX (24):

[0022] In a sixth aspect the invention pertains to a method of increasing phosphoenolpyruvate (PEP) availability in a bacterial host cell which comprises a) selecting a bacterial host cell having a PTS.sup.-/Glu.sup.- phenotype, wherein the bacterial host was originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport; b) modifying an endogenous chromosomal regulatory sequence of the selected bacterial host cell comprising transforming said selected bacterial host cell with a DNA construct comprising a promoter, wherein said DNA construct is chromosomally integrated into the selected bacterial host cell replacing an endogenous promoter which is operably linked to a nucleic acid encoding a glucose assimilation protein; c) culturing the transformed bacterial host cell under suitable conditions; and d) allowing expression of the glucose assimilation protein to obtain an altered host cell having a PTS.sup.-/Glu.sup.+ phenotype, wherein the PEP availability is increased compared to the PEP availability in a corresponding unaltered PTS bacterial host cell cultured under essentially the same culture conditions. In one embodiment the glucose assimilation protein is a galactose permease and the DNA construct comprises an exogenous promoter which replaces the endogenous promoter of the galactose permease. In another embodiment the glucose assimilation protein is a glucokinase and the DNA construct comprises an exogenous promoter which replaces the endogenous promoter of a glucokinase.

Summary of Invention Paragraph - BSTX (25):

[0023] In an eighth aspect, the invention pertains to a method for increasing the growth rate of a PTS.sup.-/Glu.sup.- bacterial host cell originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport which comprises, a) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a galactose permease in a PTS.sup.-/Glu.sup.- host cell by transforming the PTS.sup.-/Glu.sup.- host cell with a first DNA construct comprising an exogenous promoter and DNA flanking sequences corresponding to (5') upstream region of the galactose permease; b) modifying an endogenous regulatory region which is operably linked to a nucleic acid encoding a glucokinase in the PTS.sup.-/Glu.sup.- host cell by transforming the PTS.sup.-/Glu.sup.- host cell with a second DNA construct comprising an exogenous promoter and DNA flanking sequences corresponding to upstream (5') regions of the glucokinase; c)

allowing integration of the first and the second DNA constructs, wherein the first DNA construct replaces the endogenous promoter of the nucleic acid encoding the galactose permease and the second DNA construct replaces the endogenous promoter of the nucleic acid encoding the glucokinase d) culturing the transformed bacterial host cell under suitable conditions; and e) allowing expression of the galactose permease and the glucokinase from the modified regulatory regions to obtain an altered bacterial cell having an increase specific growth rate compared to the specific growth rate of a corresponding unaltered PTS bacterial host cell cultured under essentially the same culture conditions.

Brief Description of Drawings Paragraph - DRTX (3):

[0027] The following abbreviations are used in the figure and throughout the disclosure: PEP=phosphoenolpyruvate; DAHP=3-deoxy-D-arabino-heptuloso- nate 7-phosphate; DHQ=3-dehydroquinate; DHS=3-dehydroshikimate; SHK=shikimate; S3P=shikimate 3-phosphate; EPSP=5-enolpyruvyl shikimate 3-phosphate; PHE=phenylalanine; TYR=tyrosine; TRP=tryptophan; Pyk=pyruvate kinase, which is encoded by the gene pyk; and Ppc=PEP carboxylase, which is encoded by the gene ppc. Further, the following genes are illustrated for the common aromatic pathway: aroB which encodes DHQ synthase; aroD which encodes DHQ dehydratase; aroE which encodes shikimate dehydrogenase; aroL and aroK which encode shikimate kinase; aroA which encodes EPSP synthase and aroC which encodes chorismate synthase. While not specifically illustrated, one skilled in the art is aware that aroG, aroF and aroH encode the three isozymes of DAHP synthase which catalyzes the conversion of Erythrose-4P (E4P) and PEP to DAHP in E. coli. FIG. 1B illustrates the varied compounds, the production of which, may be enhanced by an increase in carbon flux and PEP availability according to the methods encompassed by the invention.

Detail Description Paragraph - DETX (8):

[0047] The phosphotransferase system (PTS) (E.C. 2.7.1.69) refers to the phosphoenolpyruvate-dependent sugar uptake system. This is a system of transporter proteins that participate in transport and phosphoenolpyruvate dependent phosphorylation of several sugars. The system is comprised of two phosphoproteins, enzyme I and HPr, which are common to all PTS carbohydrates and catalyze the transfer of a phosphoryl group from PEP to the carbohydrate specific membrane bound enzyme II and then to the carbohydrate. In some cases enzyme III is positioned between HPr and enzyme II. To distinguish the various enzymes II and enzymes III, a three letter superscript is used to indicate which carbohydrate is the preferred substrate. For example Enzyme II.sup.glc means glucose is the preferred substrate. However other substrates may be used.

Detail Description Paragraph - DETX (9):

[0048] The terms "PtsI" and "Enzyme I" refer to the phosphotransferase EC 2.7.3.9 encoded by ptsH in E. coli. The terms "HPr" and "PtsH" refer to the phosphocarrier protein encoded by ptsH in E. coli. The terms "glucose-specific IIA component", "Enzyme II.sup.glc" and "Crr" refer to EC 2.7.1.69 encoded by crr in E. coli. The PTS comprises PtsI, PtsH and Crr and functionally equivalent proteins.

Detail Description Paragraph - DETX (12):

[0051] "Increased phosphoenolpyruvate (PEP) availability" means increasing the amount of intracellular PEP which enhances carbon committed to a metabolic or productive pathway, said PEP which would otherwise have been metabolized in the PTS for phosphorylation of glucose.

Detail Description Paragraph - DETX (13):

[0052] The phrase "increasing carbon flow" means increasing the availability of carbon substrates to metabolic or productive pathways, said carbon substrate which would otherwise be diverted by the metabolism of PEP in the PTS. Carbon flow to a particular pathway can be measured by well know methods such as gas chromatography and mass spectroscopy. Carbon flow as measured by produced product may be at least 2%, 5%, 10%, 15%, 20%, 25%, 30%, or greater than the carbon flow in a corresponding PTS cell grown under essentially the same growth conditions.

Detail Description Paragraph - DETX (62):

[0100] The present invention is directed to a method for increasing carbon flow into a desired metabolic pathway of a host cell originally capable of utilizing a PTS for carbohydrate transport, said method including the steps of selecting a host cell which is effectively phenotypically PTS.sup.- and modifying at least one homologous chromosomal regulatory region, which is operably linked to a chromosomal nucleic acid which encodes a polypeptide involved in glucose assimilation, resulting in the restoration of a glucose.sup.+ phenotype and thereby increasing the carbon flow into and through a desired metabolic pathway.

Detail Description Paragraph - DETX (64):

[0102] A general review of the PTS can be found in (Postma et al., 1993, Microbiol. Rev. 57:543-594; Romano et al., 1979, J. Bacteriol. 139:93-97 and Saier et al. 1990, In: BACTERIAL ENERGETICS pp. 273-299, T. A. Krulwich, Ed. Academic Press, NY). Host cells or strains useful in the present invention include any organism capable of utilizing a PTS system for carbohydrate transport. This includes prokaryotes belonging to the genus Escherichia, Corynebacterium, Brevibacterium, Bacillus, Pseudomonas, Streptomyces, Pantoea or Staphylococcus. A list of suitable organisms is provided in Table 1. The inactivation of the PTS in any of these organisms should potentially increase carbon flux and PEP (and PEP precursor) availability in the cell for alternative metabolic routes and consequently could increase production of desired compounds (e.g., aromatics) from such cells.

Detail Description Paragraph - DETX (75):

[0113] However, to increase or redirect carbon flow to desired metabolic pathways in inactivated PTS host cells, glucose transport and phosphorylation must be deregulated or amplified.

Detail Description Paragraph - DETX (124):

[0162] It should be noted that as the host cell is cultured in conditions which create an increase in carbon flow into the aromatic pathway, it may be necessary to identify and overcome rate-limiting steps in the pathway. This methodology is available to the artisan, see, for example, U.S. Pat. Nos. 5,168,056 and 5,776,736.

Detail Description Paragraph - DETX (127):

[0165] In addition to increasing the carbon flux through the aromatic pathway, the following genes may be overexpressed in PTS.sup.-/Glu.sup.+ cells according to the invention: pps which encodes PEP synthase in E. coli (see U.S. Pat. No. 5,985,617) and talA which encodes transaldolase (Iida et al. (1993) J. Bacterial. 175:5375-5383). Further any gene encoding an enzyme that catalyzes reactions within the common aromatic pathway (for example, DAHP synthase (aroF, aroG, aroH), DHQ synthase (aroB), DHQ dehydratase (aroD), shikimate dehydrogenase (aroE), shikimate kinase (aroL, aroK), EPSP synthase (aroA) and chorismate synthase (aroC) may be amplified in the PTS.sup.-/Glu.sup.+ cells encompassed by the present invention.

Detail Description Paragraph - DETX (135):

[0173] Thus, having provided a PTS.sup.-/Glu.sup.+ strain which conserves PEP resulting in an increase in carbon flux into a metabolic pathway, such as the aromatic amino acid pathway, glycolysis, the TCA cycle, and the pentose phosphate pathway, by redirecting PEP and PEP precursors, the inventors have provided a host system which can be utilized for enhanced production of desired compounds in comparison to the production of the same compounds in a corresponding PTS host cell.

Detail Description Paragraph - DETX (187):
[0220] By constitutively expressing galP on the chromosome from the trc promoter the flux of carbon from glucose was increased into the pathway for the desired products, glycerol and 1,3-propanediol rather than into pathways to produce cell mass.

Claims Text - CLTX (1):
1. A method of increasing carbon flow into a metabolic pathway of a PTS.sup.-/Glu.sup.- bacterial host cell which was originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport comprising, a) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a glucose assimilation protein in a PTS.sup.-/Glu.sup.- host cell by transforming the PTS.sup.-/Glu.sup.- host cell with a DNA construct comprising a promoter and DNA flanking sequences corresponding to upstream (5') regions of the glucose assimilation protein; b) allowing integration of the DNA construct to restore a Glu+ phenotype; and c) culturing the transformed host cell under suitable culture conditions, wherein the carbon flow into a metabolic pathway of the transformed host cell is increased compared to the carbon flow into the same metabolic pathway in a corresponding PTS bacterial host cell cultured under essentially the same culture conditions.

Claims Text - CLTX (22):
22. A method of increasing carbon flow into a metabolic pathway of a PTS.sup.-/Glu.sup.- bacterial host cell originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport comprising, a) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a galactose permease in a PTS.sup.-/Glu.sup.- host cell by transforming the PTS.sup.-/Glu.sup.- host cell with a first DNA construct comprising a promoter and DNA flanking sequences corresponding to upstream (5') regions of the galactose permease; b) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a glucokinase in the PTS.sup.-/Glu.sup.- host cell by transforming the PTS.sup.-/Glu.sup.- host cell with a second DNA construct comprising a promoter and DNA flanking sequences corresponding to upstream (5') regions of the glucokinase; c) allowing integration of the first and the second DNA constructs, wherein the first DNA construct replaces an endogenous promoter of the nucleic acid encoding the galactose permease and the second DNA construct replaces an endogenous promoter of the nucleic acid encoding the glucokinase wherein both the galactose permease and the glucokinase are expressed in the host cell and wherein said expression results in an increase in carbon flow into a metabolic pathway of the transformed host cell compared to carbon flow into the same metabolic pathway in a corresponding unaltered PTS.sup.-/Glu.sup.- bacterial cell.

Claims Text - CLTX (26):
26. A method of restoring a Glu+ phenotype to a PTS.sup.-/Glu.sup.- bacterial host cell which was originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport comprising a) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a glucose transporter in a

PTS.sup.-/Glu.sup.- host cell by transforming the PTS.sup.-/Glu.sup.- host cell with a first DNA construct comprising a promoter and DNA flanking sequences corresponding to upstream (5') regions of the glucose transporter; b) allowing integration of the first DNA construct, wherein the first DNA construct replaces an endogenous promoter of the nucleic acid encoding the glucose transporter; and c) allowing expression of the glucose transporter, wherein said expression restores a Glu+ phenotype to the PTS.sup.-/Glu.sup.- host cell.

Claims Text - CLTX (34):

34. A method of increasing phosphoenolpyruvate (PEP) availability in a bacterial host cell comprising, a) selecting a bacterial host cell having a PTS.sup.-/Glu.sup.- phenotype, wherein the bacterial host was originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport; b) modifying an endogenous chromosomal regulatory sequence of the selected bacterial host cell comprising transforming said selected bacterial host cell with a DNA construct comprising a promoter, wherein said DNA construct is chromosomally integrated into the selected bacterial host cell replacing an endogenous promoter which is operably linked to a nucleic acid encoding a glucose assimilation protein; c) culturing the transformed bacterial host cell under suitable conditions; and d) allowing expression of the glucose assimilation protein to obtain an altered host cell having a PTS.sup.-/Glu.sup.+ phenotype, wherein the PEP availability is increased compared to the PEP availability in a corresponding unaltered PTS bacterial host cell cultured under essentially the same culture conditions.

Claims Text - CLTX (41):

41. A method for increasing the growth rate of a PTS.sup.-/Glu.sup.- bacterial host cell originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport comprising, a) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a galactose permease in a PTS.sup.-/Glu.sup.- host cell by transforming the PTS.sup.-/Glu.sup.- host cell with a first DNA construct comprising an exogenous promoter and DNA flanking sequences corresponding to (5') upstream region of the galactose permease; b) modifying an endogenous regulatory region which is operably linked to a nucleic acid encoding a glucokinase in the PTS.sup.-/Glu.sup.- host cell by transforming the PTS.sup.-/Glu.sup.- host cell with a second DNA construct comprising an exogenous promoter and DNA flanking sequences corresponding to upstream (5') regions of the glucokinase; c) allowing integration of the first and the second DNA constructs, wherein the first DNA construct replaces the endogenous promoter of the nucleic acid encoding the galactose permease and the second DNA construct replaces the endogenous promoter of the nucleic acid encoding the glucokinase d) culturing the transformed bacterial host cell under suitable conditions; and e) allowing expression of the galactose permease and the glucokinase from the modified regulatory regions to obtain an altered bacterial cell having an increase specific growth rate compared to the specific growth rate of a corresponding unaltered PTS bacterial host cell cultured under essentially the same culture conditions.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20050079482 A1

TITLE: Method for redesign of microbial production systems

PUBLICATION-DATE: April 14, 2005

INVENTOR-INFORMATION:

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APPL-NO: 10/ 929091

DATE FILED: August 26, 2004

RELATED-US-APPL-DATA:

child 10929091 A1 20040826

parent continuation-in-part-of 10616659 20030709 US PENDING

non-provisional-of-provisional 60395763 20020710 US

non-provisional-of-provisional 60417511 20021009 US

non-provisional-of-provisional 60444933 20030203 US

US-CL-CURRENT: 435/4, 702/19

ABSTRACT:

A computer-assisted method for identifying functionalities to add to an organism-specific metabolic network to enable a desired biotransformation in a host includes accessing reactions from a universal database to provide stoichiometric balance, identifying at least one stoichiometrically balanced pathway at least partially based on the reactions and a substrate to minimize a number of non-native functionalities in the production host, and incorporating the at least one stiochiometrically balanced pathway into the host to provide the desired biotransformation. A representation of the metabolic network as modified can be stored.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. patent application Ser. No. 10/616,659, filed Jul. 9, 2003 which is a conversion of: U.S. patent application Ser. No. 60/395,763, filed Jul. 10, 2002; U.S. patent application Ser. No. 60/417,511, filed Oct. 9, 2002; and U.S. patent application Ser. No. 60/444,933, filed Feb. 3, 2003, each of which is herein incorporated by reference in its entirety.

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Detail Description Paragraph - DETX (70):
[0093] is a minimum level of biomass production. The vector .nu. includes both internal and transport reactions. The forward (i.e., positive) direction of transport fluxes corresponds to the uptake of a particular metabolite, whereas the reverse (i.e., negative) direction corresponds to metabolite secretion. The uptake of glucose through the phosphotransferase system and glucokinase are denoted by .nu..sub.pts and .nu..sub.glk, respectively. Transport fluxes for metabolites that can only be secreted from the network are members of M.sub.secr.sub..sub.--.s- ub.only. Note also that the complete set of reactions M is subdivided into reversible M.sub.rev and irreversible M.sub.irrev reactions. The cellular objective is often assumed to be a drain of biosynthetic precursors in the ratios required for biomass formation (Neidhardt and Curtiss, 1996). The fluxes are reported per 1 gDW.multidot.hr such that biomass formation is expressed as g biomass produced/gDW.multidot.hr or 1/hr.

Detail Description Paragraph - DETX (88):
[0109] Which reactions, if any, that could be removed from the E. coli K-12 stoichiometric model (Edwards & Palsson, 2000) so as the remaining network produces succinate or lactate whenever biomass maximization is a good descriptor of flux allocation were identified. A prespecified amount of glucose (10 mmol/gDW.multidot.hr), along with unconstrained uptake routes for inorganic phosphate, oxygen, sulfate, and ammonia are provided to fuel the metabolic network. The optimization step could opt for or against the phosphotransferase system, glucokinase, or both mechanisms for the uptake of glucose. Secretion routes for acetate, carbon dioxide, ethanol, formate, lactate and succinate are also enabled. Note that because the glucose uptake rate is fixed, the biomass and product yields are essentially equivalent to the rates of biomass and product production, respectively. In all cases, the OptKnock procedure eliminated the oxygen uptake reaction pointing at anaerobic growth conditions consistent with current succinate (Zeikus et al., 1999) and lactate (Datta et al., 1995) fermentative production strategies.

Detail Description Paragraph - DETX (90):
[0111] A less intuitive strategy was identified for mutant C which focused on inactivating two PEP consuming reactions rather than eliminating competing byproduct (i.e., ethanol, formate, and lactate) production mechanisms. First, the phosphotransferase system was disabled requiring the network to rely exclusively on glucokinase for the uptake of glucose. Next, pyruvate kinase was removed leaving PEP carboxykinase as the only central metabolic reaction capable of draining the significant amount of PEP supplied by glycolysis. This strategy, assuming that the maximum biomass yield could be attained, resulted in a succinate yield approaching 88% of the theoretical maximum. In addition, there was significant succinate production for every attainable biomass yield, while the maximum theoretical yield of succinate is the same as that for the wild-type strain.

Detail Description Table CWU - DETL (1):
1TABLE I Biomass and chemical yields for various gene knockout strategies identified by OptKnock. The reactions and corresponding enzymes for each knockout strategy are listed. The maximum biomass and corresponding chemical yields are provided on a basis of 10 mmol/hr glucose fed and 1 gDW of cells. The rightmost column provides the chemical yields for the same basis assuming a minimal redistribution of metabolic fluxes from the wild-type (undeleted) E. coli network (MOMA assumption). For the 1,3-propanediol case, glycerol secretion was disabled for both knockout strategies. max .nu..sub.biomass min .SIGMA.(84.sub.0 - .nu.).sup.2 Succinate Biomass Succinate Succinate ID Knockouts Enzyme (1/hr) (mmol/hr) (mmol/hr) Wild "Complete network" 0.38 0.12 0 A 1 COA + PYR ACCOA + FOR Pyruvate formate lyase 0.31 10.70 1.65 2

NADH + PYR LAC + NAD Lactate dehydrogenase B 1 COA + PYR ACCOA + FOR Pyruvate formate lyase 0.31 10.70 4.79 2 NADH + PYR LAC + NAD Lactate dehydrogenase 3 ACCOA + 2 NADH COA + ETh + 2 NAD Acetaldehyde dehydrogenase C 1 ADP + PEP ATP + PYR Pyruvate kinase 0.16 15.15 6.21 2 ACTP + ADP AC + ATP or Acetate kinase ACCOA + Pi ACTP + COA Phosphotransacetylase 3 GLC + PEP G6P + PYR Phosphotransferase system max .nu..sub.biomass min .SIGMA.(84.sub.0 - .nu.).sup.2 Lactate Biomass Lactate Lactate ID Knockouts Enzyme (1/hr) (mmol/hr) (mmol/hr) Wild "Complete network" 0.38 0 0 A 1 ACTP + ADP AC + ATP or Acetate kinase 0.28 10.46 5.58 ACCOA + Pi ACTP + COA Phosphotransacetylase 2 ACCOA + 2 NADH COA + ETh + 2 NAD Acetaldehyde dehydrogenase B 1 ACTP + ADP AC + ATP or Acetate kinase 0.13 18.00 0.19 ACCOA + Pi ACTP + COA Phosphotransacetylsse 2 ATP + F6P ADP + FDP or Phosphofructokinase FDP T3P1 + T3P2 Fructose-1,6-bisphosphatate aldolase C 1 ACTP + ADP AC + ATP or Acetatekinase 0.12 18.13 10.53 ACCOA + Pi ACTP + COA Phosphotransacetylase 2 ATP + F6P ADP + FDP or Phosphofructokinase FDP T3P1 + T3P2 Fructose-1,6-bisphosphatate aldolase 3 ACCOA + 2 NADH COA + ETH + 2 NAD Acetaldehyde dehydrogenase 4 GLC + ATP G6P + PEP Glucokinase max .nu..sub.biomass min .SIGMA.(84.sub.0 - .nu.).sup.2 1,3-Propanediol Biomass 1,3-PD 1,3-PD ID Knockouts Enzyme (1/hr) (mmol/hr) (mmol/hr) Wild "Complete network" 1.06 0 0 A 1 FDP F6P + Pi or Fructose-1,6-bisphosphatase 0.21 9.66 8.66 FDP T3P1 + T3P2 Fructose-1,6-bisphosphste aldolase 2 13PDG + ADP 3PG + ATP or Phosphoglycerate kinase NAD + Pi + T3P1 13PDG + NADH Glyceraldehyde-3-phosphate debydrogenase 3 GL + NAD GLAL + NADH Aldebyde dehydrogenase B 1 T3P1 T3P2 Triosphosphate isomerase 0.29 9.67 9.54 2 G6P + NADP D6PGL + NADPH or Glucose 6-phosphate-1-dehydrogenase D6PGL D6PGC 6-Phosphoglucooolactona- se 3 DR5P ACAL + T3P1 Deoxyribose-phosphate aldolase 4 GL + NAD GLAL + NADH Aldehyde dehydrogenase

PGPUB-DOCUMENT-NUMBER: 20040261147

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040261147 A1

TITLE: High level production of arbutin in green plants and microbes

PUBLICATION-DATE: December 23, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 462162

DATE FILED: June 16, 2003

US-CL-CURRENT: 800/278, 435/468 , 435/471 , 435/488

ABSTRACT:

This invention relates to methods and materials to produce hydroquinone glucoside in genetically modified green plants and microorganisms.

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Detail Description Paragraph - DETX (71):

[0092] All co-factors, co-enzymes and co-substrates required for these pathways (such as FAD, NADH, O.sub.2, and UDPG) are present in the E. coli cytoplasm. E. coli strains with elevated levels of chorismate resulting from increased flux of carbon into the shikimate pathway have been disclosed (Berry et al., Trends Biotech, 14(7):250-256 (1996); Bongaerts et al., Metabolic Engineering, 3(4):289-300 (2001); Tatarko and Romeo T., Curr. Microbiol., 43(1):26-32 (2001); U.S. Pat. No. 6,210,937 B1; U.S. Pat. No. 5,776,736 A; and WO 73484 A1) and can be used to produce arbutin from glucose using the molecular tools described below.

Detail Description Paragraph - DETX (108):

[0129] Some degree of improvement has been achieved in this area by including in the chloroplast targeting sequence not only the transit peptide and the scissile bond, but also a small portion of the mature N-terminus of the transit peptide donor. Indeed, this approach has worked both in vivo and in vitro ((Van den Broeck et al., supra; Schreier et al., supra; Wasmann et al., supra; EP 0189707; U.S. Pat. No. 5,728,925; and U.S. Pat. No. 5,717,084)) for another bacterial protein, namely, neomycin phosphotransferase II (NPT-II). Thus, a chimeric protein consisting of the transit peptide of the Rubisco small subunit precursor plus the first 22 residues of mature Rubisco fused to the N-terminus of NPT-II was taken up by chloroplasts much better than a similar construct that only contained the transit peptide and scissile bond. However, this strategy is not foolproof as it is still associated with a high degree of unpredictability that is inextricably linked to the passenger protein. This is most readily seen in the literature attempts to target CPL to chloroplasts. For example, Sommer et al. (Plant Cell Physiol., 39(11):1240-1244 (1998))

describe an analogous artificial fusion protein comprising the CPL gene product fused at its N-terminus to the transit peptide and first 21 amino acid residues of the Rubisco small subunit (e.g., "TP21 UbiC"). This modification was undertaken to improve chloroplast uptake and processing, but the cells that contained the original construct, TP-UbiC, unexpectedly had much higher levels of both CPL enzyme activity and pHBA glucosides. Thus, application of the Wasmann et al. (supra) teaching had a detrimental effect when applied with a different protein.

Detail Description Paragraph - DETX (147):

[0167] The molecular identification and isolation of the pHBA 1-hydroxylase gene described by Applicants also enables arbutin production in microbial systems such as E. coli (described in detail in Examples 7 and 8). The pHBA 1-hydroxylase gene is expressed in E. coli cells in combination with a chorismate pyruvate-lyase gene and a suitable glucosyltransferase gene (Examples 6 and 8). Expressing these three enzymes in E. coli provides a route to arbutin from a cheap, fermentable carbon source, such as glucose, by creating a three-step pathway from chorismate to arbutin (FIG. 1). Those skilled in the art will recognize that all cofactors, coenzymes and co-substrates required for this pathway such as FAD, NADH, O.sub.2, and UDP-glucose (UDPG) are present in the E. coli cytoplasm. Moreover E. coli strains with elevated levels of chorismate resulting from increased flux of carbon into the shikimate pathway have been disclosed (Berry et al., Trends Biotech., 14(7):250-256 (1996); Bongaerts et al., Metabolic Engineering, 3(4):289-300 (2001); Tatarko and Romeo, Current Microbiology, 43(1):26-32 (2001); U.S. Pat. No. 6,210,937; US 5776736; and WO 73484 A1) and can be used to produce arbutin from glucose using the molecular tools described below.

PGPUB-DOCUMENT-NUMBER: 20040209337

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040209337 A1

TITLE: Synthesis of 1,2,3,4-tetrahydroxybenzenes from biomass-derived carbon

PUBLICATION-DATE: October 21, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 838551

DATE FILED: May 4, 2004

RELATED-US-APPL-DATA:

child 10838551 A1 20040504

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parent-patent 6750049 US

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parent a-371-of-international PCT/US00/06808 20000316 WO PENDING

child 09937243 20020507 US

parent continuation-of 09274732 19990323 US ABANDONED

US-CL-CURRENT: 435/156, 568/763

ABSTRACT:

A bioengineered synthesis scheme for the production of 1,2,3,4-tetrahydroxybenzene from a carbon source is provided. Methods of producing 1,2,3,4-tetrahydroxybenzene acid from a carbon source based on the synthesis scheme are also provided. Methods are also provided for converting 1,2,3,4-tetrahydroxybenzene to 1,2,3-trihydroxybenzene by catalytic hydrogenation.

RELATED APPLICATIONS

[0001] The present application is a divisional of U.S. application Ser. No. 09/937,243, filed Sep. 21, 2001, which is a national phase continuation of PCT international publication WO 00/56911 filed Mar. 16, 2000, which is a Continuation-in-Part of U.S. application Ser. No. 09/274,732 filed Mar. 23, 1999, which is hereby expressly incorporated by reference.

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Detail Description Paragraph - DETX (8):

[0024] In one embodiment, a recombinant E. coli microbe is employed in the methods of the present invention. In a preferred embodiment, the E. coli comprises a non-functional serA locus. This recombinant E. coli, designated, JWF1, may further comprise a plasmid carrying an INO1 gene insert and a serA gene insert. The INO1 gene encodes myo-inositol-1-phosphate synthase which converts glucose-6-phosphate to myo-inositol-1-phosphate. In a preferred embodiment, the INO1 gene is from Saccharomyces cerevisiae. Overexpression of myo-inositol-1-phosphate synthase will increase carbon flow into the myo-inositol pathway. This recombinant microbe is capable of converting glucose to myo-inositol.

Detail Description Paragraph - DETX (10):

[0026] In a preferred embodiment, the recombinant E. coli comprises plasmid pAD1.88A carrying an INO1 gene insert and a serA gene insert. As described above, the INO1 gene insert encodes myo-inositol-1-phosphate synthase which converts glucose-6-phosphate to myo-inositol-1-phosphate, thus increasing the carbon flow into the myo-inositol pathway. Due to a mutation in the E. coli genomic serA locus required for L-serine biosynthesis, growth in minimal salts medium and plasmid maintenance follows from expression of plasmid-localized serA. The serA insert thus allows microbial growth in minimal salts medium, distinguishing the microbes containing the plasmid from non-plasmid containing microbes.

Detail Description Paragraph - DETX (26):

[0040] Synthesis of myo-inositol by E. coli JWF1/pAD1.88A begins with D-glucose uptake and conversion to D-glucose-6-phosphate catalyzed by the E. coli phosphotransferase system (Postma, P. W. et al., In Escherichia coli and Salmonella, 2nd ed., Neidhardt, F. C. et al., Eds., ASM: Washington, Vol. 1, p. 1149 (1996)) where phosphoenolpyruvate is the source of the transferred phosphoryl group. D-Glucose-6-phosphate then undergoes cyclization to myo-inositol 1-phosphate catalyzed by myo-inositol-1-phosphate synthase. This enzyme activity, which results from expression of the Saccharomyces cerevisiae INO1 gene (Dean-Johnson, M. et al., J. Biol. Chem. 264:1274 (1989)) on plasmid pAD1.88A, varied significantly (0.022, 0.043, 0.018, and 0.009 .mu.mol/min/mg at 18 h, 30 h, 42 h, and 54 h, respectively) over the course of the fermentation.

PGPUB-DOCUMENT-NUMBER: 20040203093

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040203093 A1

TITLE: NUCLEOTIDE SEQUENCE OF THE HAEMOPHILUS INFLUENZAE RD GENOME, FRAGMENTS THEREOF, AND USES THEREOF

PUBLICATION-DATE: October 14, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 158865

DATE FILED: June 3, 2002

RELATED-US-APPL-DATA:

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child 09557884 20000425 US

parent continuation-of 08476102 19950607 US GRANTED

parent-patent 6355450 US

child 08476102 19950607 US

parent continuation-in-part-of 08426787 19950421 US ABANDONED

US-CL-CURRENT: 435/69.1, 435/320.1 , 435/325 , 435/6 , 536/23.2 , 702/20

ABSTRACT:

The present invention provides the sequencing of the entire genome of Haemophilus influenzae Rd, SEQ ID NO:1. The present invention further provides the sequence information stored on computer readable media, and computer-based systems and methods which facilitate its use. In addition to the entire genomic sequence, the present invention identifies over 1700 protein encoding fragments of the genome and identifies, by position relative to a unique Not I restriction endonuclease site, any regulatory elements which modulate the expression of the protein encoding fragments of the Haemophilus genome.

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Detail Description Paragraph - DETX (204):

[0230] In order to survive as a free living organism, H. influenaze must produce energy in the form of ATP via fermentation and/or electron transport. As a facultative anaerobe, H. influenaze Rd is known to ferment glucose, fructose, galactose, ribose, xylose and fucose (Dorocicz et al., J. Bacteriol. 175:7142 (1993)). The genes identified in Table 1(a) indicate that transport systems are available for the uptake of these sugars via the phosphoenolpyruvate-phosphotransferase system (PTS), and via non-PTS mechanisms. Genes that specify the common phosphate-carriers Enzyme I and Hpr (ptsI and ptsH) of the PTS system were identified as well as the glucose specific crr gene. The ptsH, ptsI, and crr genes constitute the pts operon. We have not however identified the gene encoding membrane-bound glucose specific Enzyme II. The latter enzyme is required for transport of glucose by the PTS system. A complete FITS system for fructose was identified.

Detail Description Table CWU - DETL (10):

HI1389 1480494 1480988 ferritin like protein (rsgA) [Escherichia coli] 57.3 73.8 164 HI0363 385804 384887 iron(III) dicitrate transport ATP-binding protein FECE [Escherichia coli] 35.9 56.4 220 HI1274 1347324 1347861 iron(III) dicitrate transport system permease protein (fecD) [Escherichia 36.0 64.0 255 coli] HI1037 1099321 1100265 magnesium and cobalt transport protein (corA) [Escherichia coli] 70.3 84.8 316 HI0097 103798 104679 major ferric iron binding protein precursor (fbp) [Neisseria gonorrhoeae] 69.7 82.3 293 HI1051 1114308 1114635 mercuric transport protein (merT) [Pseudomonas aeruginosa] 25.0 55.2 99 HI1052 1114651 1114926 mercury scavenger protein (merP) [Pseudomonas fluorescens] 29.3 45.7 91 HI0294 327396 327193 mercury scavenger protein (merP) [Psudomonas fluorescens] 32.8 67.2 67 HI1531 1594953 1594219 molybdate-binding periplasmic protein precursor (modB) [Azotobacter 21.7 43.0 245 vinelandii] HI0226 254880 253681 NA(+)/H(+) antiporter 1 (nhaA) [Escherichia coli] 52.6 74.6 380 HI0429 448992 450557 Na+/H+ antiporter (nhaB) [Escherichia coli] 70.6 87.5 501 HI1110 1171933 1170530 Na+/H+ antiporter (nhaC) [Bacillus firmus] 37.5 62.0 382 HI0098 104899 106317 periplasmic-binding-protein-dependent iron transport protein (sfuB) 38.1 59.5 457 [Serratia marcescens] HI1479 1558763 1558167 periplasmic-binding-protein-dependent iron transport protein (sfuC) 39.9 58.0 197 [Serratia marcescens] HI0913 964424 966276 potassium efflux system (kefC) [Escherichia coli] 40.9 65.7 594 HI0292 326934 324769 potassium/copper-transportING ATPase A (copA) [Enterococcus faecalis] 42.9 64.4 723 HI1355 1429787 1428276 sodium/proline symporter (proline permease) (putP) [Escherichia coli] 62.8 79.1 489 HI0252 283326 282517 tonB protein (tonB) [Haemophilus influenzae] 96.2 98.5 261 HI0627 664922 666362 TRK system potassium uptake protein (trkA) [Escherichia coli] 65.8 83.4 458 Carbohydrates, organic alcohols & acids HI0020 22097 20661 2-oxoglutarate/malate translocator (SODiT1) [Spinacia oleracea] 35.8 59.6 452 HI0824 872894 873940 D-galactose-binding periplasmic protein (mglB) [Escherichia coli] 67.6 81.2 329 HI1113 1176024 1174516 D-xylose transport ATP-binding protein (xylG) [Escherichia coli] 71.5 85.8 501 HI1114 1177073 1176078 D-xylose-binding periplasmic protein (rbsB) [Escherichia coli] 76.0 88.4 328 HI1718 1785024 1783300 enzyme I (ptsI) [Salmonella typhimurium] 70.2 84.3 574 HI0182 194818 193967 formate transporter (formate channel) [Escherichia coli] 53.2 73.4 263 HI0450 471781 470285 fructose-permease IIA/FPR component (fruB) [Escherichia coli] 51.5 68.3 374 HI0448 469337 467670 fructose-permease IIBC component (fruA) [Escherichia coli] 57.2 72.2 552 HI0614 643282 642851 fucose operon protein (fucU) [Escherichia coli] 66.3 80.0 94 HI0692 733673 734464 glpF protein (glpF) [Escherichia coli] 73.6 87.2 258 HI1019 1080518 1081194 glpF protein (glpF) [Escherichia coli] 30.6 54.6 208 HI1017 1078404 1079867 gluconate permease (gntP) [Bacillus subtilis] 29.1 56.4 442 HI1717 1783237 1782740 glucose phosphotransferase enzyme III-glc (crr) [Escherichia coli] 73.2 83.3 169 HI0688 729474 730914 glycerol-3-phosphatase transporter (glpT) [Escherichia coli] 64.5 78.9 445

HI0504 517869 519347 high affinity ribose transport protein (rbsA) [Escherichia coli] 71.1 85.4 494 HI0505 519363 520331 high affinity ribose transport protein (rbsC) [Escherichia coli] 68.0 86.5 303 HI0503 517436 517852 high affinity ribose transport protein (rbsD) [Escherichia coli] 59.0 78.4 139 HI0612 642139 640856 L-fucose permease (fucP) [Escherichia coli] 35.6 57.9 413 HI1221 1288578 1286983 L-lactate permease (lctP) [Escherichia coli] 30.2 53.9 532 HI1735 1802527 1801757 lactam utilization protein (lamB) [Emencella nidulans] 41.3 60.3 130 HI0825 874009 875526 mglA protein (mglA) [Escherichia coli] 73.9 84.6 506 HI0826 875546 876553 mglC protein (mglC) [Escherichia coli] 79.2 90.2 336 HI0506 520354 521229 periplasmic ribose-binding protein (rbsB) [Escherichia coli] 73.9 86.6 291 HI1719 1785361 1785107 phosphohistidinoprotein- -hexose phosphotransferase (ptsH) [Escherichia 77.6 88.2 85 coli] HI0830 878480 878773 potassium channel homolog (kch) [Escherichia coli] 67.7 80.2 96 HI0154 170140 168807 putative aspartate transport protein (dcuA) [Escherichia coli] 46.4 69.9 436 HI0748 803856 805175 putative aspartate transport protein (dcuA) [Escherichia coli] 42.6 70.1 435 HI1112 1174509 1173385 nbose transport permease protein (xylH) [Escherichia coli] 69.8 84.1 371 HI1696 1759373 1760743 sodium- and chloride-dependent GABA transporter [Homo sapiens] 29.3 52.6 471 HI0738 790926 789403 sodium-dependent noradrenaline transporter [Homo sapiens] 31.1 54.2 523 Nucleosides, purines & pyrimidines HI1089 1151815 1151024 ribonucleotide transport ATP-binding protein (mkl) [Mycobacterium leprae] 42.2 61.5 244 HI1230 1296319 1295078 uracil permease (ursA) [Escherichia coli] 37.2 61.6 400 Anions HI1104 1164213 1165028 cysteine synthetase (cysZ) [Escherichia coli] 53.7 76.3 190 HI1697 1761825 1760773 hydrophilic membrane-bound protein (modC) [Escherichia coli] 55.9 74.5 263 HI1698 1762501 1761815 hydrophobic membrane-bound protein (modB) [Escherichia coli] 65.9 84.8 223 HI1384 1477430 1476585 integral membrane protein (pstA) [Escherichia coli] 59.6 77.6 272 HI0356 380045 380764 nitrate transporter ATPase component (nasD) [Klebsiella pneumoniae] 34.9 57.8 254 HI1383 1475710 1476584 peripheral membrane protein B (pstB) [Escherichia coli] 77.0 86.8 256 HI1385 1478379 1477435 peripheral membrane protein C (pstC) [Escherichia coli] 57.3 78.7 300 HI1386 1479246 1478473 periplasmic phosphate-binding protein (pstS) [Escherichia coli] 49.8 67.7 256 HI1387 1479247 1479929 periplasmic phosphate-binding protein (pstS) [Escherichia coli] 63.8 75.4 69 HI1610 1669474 1670733 phosphate permease(YBR296C) [Saccharomyces cerevisiae] 35.6 60.0 551 Other HI0060 62564 60804 ATP dependent translocator homolog (msbA) [Haemophilus influenzae] 100.0 100.0 458 HI0623 653683 662010 ATP-binding protein (abc)

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TITLE: Materials and methods for the efficient production of acetate and other products

PUBLICATION-DATE: August 5, 2004

INVENTOR-INFORMATION:

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RELATED-US-APPL-DATA:

non-provisional-of-provisional 60424372 20021106 US

US-CL-CURRENT: 435/69.1, 435/252.3

ABSTRACT:

The subject invention provides materials and methods wherein unique and advantageous combinations of gene mutations are used to direct carbon flow from sugars to a single product. The techniques of the subject invention can be used to obtain products from native pathways as well as from recombinant pathways. In preferred embodiments, the subject invention provides new materials and methods for the efficient production of acetate and pyruvic acid.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Serial No. 60/424,372, filed Nov. 6, 2002.

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Detail Description Paragraph - DETX (23):

[0061] Genetically modified E. coli W3110 was developed to produce acetic acid as the primary product from glucose during aerobic growth using only mineral salts as nutrients. The resulting biocatalyst (TC36) contains multiple chromosomal alterations (FIG. 1) that direct carbon flow to acetate and minimize carbon loss to cell mass, CO.sub.2, and alternative products. Strain TC36 is devoid of plasmids and antibiotic resistance genes, both potential advantages for commercial use. The subject invention provides an additional derivative of Escherichia coli W3110 as a new biocatalyst for the production of homo-acetate. Acetate production by this new strain (TC36) approaches the theoretical maximum of two acetate per glucose due to the disruption of oxidative phosphorylation.

Detail Description Paragraph - DETX (24):

[0062] Chromosomal deletions were used instead of point mutations to maximize stability. All antibiotic resistance genes and auxotrophic requirements were eliminated to permit growth in simple mineral salts medium. During oxidative metabolism, up to half of the substrate carbon can be converted to roughly equal amounts of cell mass and CO.sub.2 (Contiero, J., C. Beatty, S. Kumar, C. L. DeSanti, W. R. Strohl, and A. Wolfe, 2000 "Effects of mutations in acetate metabolism on high-cell-density growth of Escherichia coli" J. Ind. Microbiol. 24:421-430; Neidhardt, F. C., J. L. Ingraham, and M. Schaechter, 1990 "Physiology of the bacterial cell: A molecular approach" Sinauer Associates, Inc., Sunderland, Mass.; Varma, A., B. W. Boesch, and B. O. Palsson, 1993 "Stoichiometric interpretation of Escherichia coli glucose catabolism under various oxygenation rates" Appl. Environ. Microbiol. 59:2465-2473) with minimal carbon flow into alternative products, such as acetate.

Detail Description Paragraph - DETX (28):

[0066] With additional mutations in fermentation pathways, further metabolism of pyruvate was limited primarily to small biosynthetic needs and conversion to acetyl.about.CoA by the pyruvate dehydrogenase complex. Although pyruvate dehydrogenase is activated by low NADH, acetyl.about.CoA production may be limited by the availability of free CoA (note pyruvate accumulation in TC36 broth between 9 h and 15 h; FIG. 4C). Resulting rises in pyruvate pools would serve as an allosteric activator of phosphotransferase (Suzuki, T., 1969 "Phosphotransacetylase of Escherichia coli B, activation by pyruvate and inhibition by NADH and certain nucleotides" Biochim. Biophys. Acta 191:559-569), the first committed step for acetate production from acetyl.about.CoA. Gratuitous ATP hydrolysis by F1-ATPase should ensure the availability of ADP for the final step in acetate production catalyzed by acetate kinase (FIG. 1). Excess pyruvate can also be directly oxidized to acetate by pyruvate oxidase (poxB), an enzyme that is induced during the latter stages of growth and by environmental stress (Chang, Y.-Y., A.-Y. Wang, and J. E. Cronan, Jr., 1994 "Expression of Escherichia coli pyruvate oxidase (PoxB) depends on the sigma factor enocoded by the rpoS (katF) gene" Mol. Microbiol. 11:1019-1028). This enzyme may also contribute to acetate production by TC36.

Detail Description Paragraph - DETX (83):

[0114] Pyruvate levels in the broth of TC36 increased (16 mM at 12 h) during the transition stage (FIG. 4C). Although this pyruvate was subsequently metabolized, the excretion of pyruvate indicates that glucose uptake and glycolysis per se may not be limiting for acetate production. Because of the various mutations in TC36, metabolism of pyruvate is limited primarily to small biosynthetic needs and conversion to acetyl.about.CoA by the pyruvate dehydrogenase complex (FIG. 1). Although pyruvate dehydrogenase is activated by low NADH, acetyl.about.CoA production may be limited by the availability of free CoA. Resulting rises in pyruvate pools (FIG. 4C), would serve as an allosteric activator of phosphotransferase (Suzuki, T., 1969 "Phosphotransacetylase of Escherichia coli B, activation by pyruvate and inhibition by NADH and certain nucleotides" Biochim. Biophys. Acta 191:559-569), since phosphotransferase (pta) is the first committed step for acetate production from acetyl.about.CoA (FIG. 1). Gratuitous ATP hydrolysis by F.sub.1-ATPase (FIG. 1C) should ensure the availability of ADP for the final step in acetate production catalyzed by acetate kinase (ackA) (FIG. 1). Excess pyruvate can also be directly oxidized to acetate by pyruvate oxidase (poxB), an enzyme that is induced during the latter stages of growth and by environmental stress (Chang, Y.-Y., A.-Y. Wang, and J. E. Cronan, Jr., 1994 "Expression of Escherichia coli pyruvate oxidase (PoxB) depends on the sigma factor enocoded by the rpoS (katF) gene" Mol. Microbiol. 11:1019-1028). Thus, pyruvate oxidase (poxB) may also contribute to acetate production by TC36.

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TITLE: Regulating metabolism by modifying the level of trehalose-6-phosphate

PUBLICATION-DATE: May 13, 2004

INVENTOR-INFORMATION:

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RELATED-US-APPL-DATA:

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FOREIGN-APPL-PRIORITY-DATA:

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NL	EP96201225-8	1996NL-EP96201225-8	May 3, 1996
NL	EP96202128.3	1996NL-EP96202128.3	July 26, 1996
NL	EP96202395.8	1996NL-EP96202395.8	August 29, 1996

US-CL-CURRENT: 800/284, 435/468

ABSTRACT:

Method for the inhibition of carbon flow in the glycolytic direction in a cell by increasing the intracellular availability of trehalose-6-phosphate.

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Abstract Paragraph - ABTX (1):
Method for the inhibition of carbon flow in the glycolytic direction in a cell by increasing the intracellular availability of trehalose-6-phosphate.

Summary of Invention Paragraph - BSTX (14):
[0012] It has now been found that modification of the development and/or composition of cells, tissue and organs in vivo is possible by introducing the enzyme trehalose-6-phosphate synthase (TPS) and/or trehalose-6-phosphatase phosphate (TPP) thereby inducing a change in metabolic pathways of the saccharide trehalose-6-phosphate IT-6-P) resulting in an alteration of the

intracellular availability of T-6-P. Introduction of TPS thereby inducing an increase in the intracellular concentration of T-6-P causes inhibition of carbon flow in the glycolytic direction, stimulation of the photosynthesis, inhibition of growth stimulation of sink-related activity and an increase in storage of resources. Introduction of TPP thereby introducing a decrease in the intracellular concentration of T-6-P causes stimulation of carbon flow in the glycolytic direction, increase in biomass and a decrease in photosynthetic activity.

Brief Description of Drawings Paragraph - DRTX (2):

[0030] FIG. 1. Schematic representation of plasmid pVDH275 harbouring the neomycin-phosphotransferase gene (NPTII) flanked by the 35S cauliflower mosaic virus promoter (P35S) and terminator (T35S) as a selectable marker; an expression cassette comprising the pea plastocyanin promoter (pPCpea) and the nopaline synthase terminator (Tnos); right (RB) and left (LB) T-DNA border sequences and a bacterial kanamycin resistance (KanR) marker gene.

Detail Description Paragraph - DETX (14):

[0078] Generation of the "plenty" signal by decreasing the intracellular concentration of trehalose-6-phosphate through expression of the enzyme TPP (or inhibition of the enzyme TPS) will signal all cell systems to increase glycolytic carbon flow and inhibit photosynthesis. This is nicely shown in the experimental part, where, for instance in Experiment 2 transgenic tobacco plants are described in which the enzyme TPP is expressed having increased leaf size, increased branching and a reduction of the amount of chlorophyll.

Detail Description Paragraph - DETX (51):

[0115] To select or screen for transformed cells, it is preferred to include a marker gene linked to the plant expressible gene according to the invention to be transferred to a plant cell. The choice of a suitable marker gene in plant transformation is well within the scope of the average skilled worker; some examples of routinely used marker genes are the neomycin phosphotransferase genes conferring resistance to kanamycin (EP-B 131 623), the glutathion-S-transferase gene from rat liver conferring resistance to glutathione derived herbicides (EP-A 256 223), glutamine synthetase conferring upon overexpression resistance to glutamine synthetase inhibitors such as phosphinothricin (WO 87/05327), the acetyl transferase gene from Streptomyces viridochromogenes conferring resistance to the selective agent phosphinothricin (EP-A 275 957), the gene encoding a 5-enolshikimate-3-phosphate synthase (EPSPS) conferring tolerance to N-phosphonomethylglycine, the bar gene conferring resistance against Bialaphos (e.g. WO 91/02071) and the like. The actual choice of the marker is not crucial as long as it is functional (i.e. selective) in combination with the plant cells of choice.

Claims Text - CLTX (3):

3. Method for the inhibition of carbon flow in the glycolytic direction in a cell by increasing the intracellular availability of trehalose-6-phosphate.

PGPUB-DOCUMENT-NUMBER: 20040073976

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040073976 A1

TITLE: Expression of fructuse 1,6 bisphosphate aldolase in transgenic plants

PUBLICATION-DATE: April 15, 2004

INVENTOR-INFORMATION:

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parent-patent 6663906 US

child 10164204 20020606 US

parent division-of 09098219 19980616 US GRANTED

parent-patent 6441277 US

non-provisional-of-provisional 60049955 19970617 US

US-CL-CURRENT: 800/284, 435/189 , 435/320.1 , 435/419 , 536/23.2

ABSTRACT:

Fructose-1,6-bisphosphate aldolase (FDA) is an enzyme reversibly catalyzing the reaction converting triosephosphate into fructose-1,6-bisphosphate. In the leaf, this enzyme is located in the chloroplast (starch synthesis) and the cytosol (sucrose biosynthesis). Transgenic plants were generated that express the E. coli fda gene in the chloroplast to improve plant yield by increasing leaf starch biosynthetic ability in particular and sucrose production in general. Leaves from plants expressing the fda transgene showed a significantly higher starch accumulation, as compared to control plants expressing the null vector, particularly early in the photoperiod, but had lower leaf sucrose. Transgenic plants also had a significantly higher root mass. Furthermore, transgenic potatoes expressing fda exhibited improved uniformity of solids.

[0001] This application is based on U.S. Provisional Application Serial No. 60/049,995, filed Jun. 17, 1997.

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Summary of Invention Paragraph - BSTX (26):
[0024] First, increasing the expression of the FDA enzyme in the chloroplast would increase the flow of carbon through the Calvin Cycle and increase atmospheric carbon assimilation during early photoperiod. This would result in an increase in photosynthetic efficiency and an increase in chloroplast starch production (a leaf carbon storage form degraded during periods when photosynthesis is low or absent). Both of these responses would lead to an increase in sucrose production by the leaf and a net increase in carbon export during a given photoperiod. This increase in source capacity is a desirable trait in crop plants and would lead to increased plant growth, storage ability, yield, vigor, and stress tolerance.

Summary of Invention Paragraph - BSTX (28):
[0026] Third, expression of FDA in sink tissues can show several desirable traits, such as increased amino acid and/or fatty acid pools via increases in carbon flux through glycolysis (and thus pyruvate levels) in seeds or other sinks and increased starch levels as result of increased production of glucose 6-phosphate in seeds, roots, stems, and tubers where starch is a major storage nonstructural carbohydrate (reverse glycolysis). This increase in sink strength is a desirable trait in crop plants and would lead to increased plant growth, storage ability, yield, vigor, and stress tolerance.

Detail Description Paragraph - DETX (2):
[0038] This invention is directed to a method for producing plant cells and plants demonstrating an increased or improved growth and development, yield, quality, starch storage uniformity, vigor, and/or stress tolerance. The method utilizes a DNA sequence encoding an fda (fructose 1,6 bisphosphate aldolase) gene integrated in the cellular genome of a plant as the result of genetic engineering and causes expression of the FDA enzyme in the transgenic plant so produced. Plants that overexpress the FDA enzyme exhibit increased carbon flow through the Calvin Cycle and increased atmospheric carbon assimilation during early photoperiod resulting in an increase in photosynthetic efficiency and an increase in starch production. Thus, such plants exhibit higher levels of sucrose production by the leaf and the ability to achieve a net increase in carbon export during a given photoperiod. This increase in source capacity leads to increased plant growth that in turn generates greater biomass and/or increases the size of the sink and ultimately providing greater yields of the transgenic plant. This greater biomass or increased sink size may be evidenced in different ways or plant parts depending on the particular plant species or growing conditions of the plant overexpressing the FDA enzyme. Thus, increased size resulting from overexpression of FDA may be seen in the seed, fruit, stem, leaf, tuber, bulb or other plant part depending upon the plant species and its dominant sink during a particular growth phase and upon the environmental effects caused by certain growing conditions, e.g. drought, temperature or other stresses. Transgenic plants overexpressing FDA may therefore have increased carbon assimilation, export and storage in plant source and sink organs, which results in growth, yield, and uniformity and quality improvements.

Detail Description Paragraph - DETX (3):
[0039] Plants overexpressing FDA may also exhibit desirable quality traits such as increased production of starch, oils and/or proteins depending upon the plant species overexpressing the FDA. Thus, overexpression of FDA in a particular plant species may affect or alter the direction of the carbon flux thereby directing metabolite utilization and storage either to starch

production, protein production or oil production via the role of FDA in the glycolysis and gluconeogenesis metabolic pathways.

Detail Description Paragraph - DETX (36):
[0066] A recombinant DNA molecule of the invention typically includes a selectable marker so that transformed cells can be easily identified and selected from non-transformed cells. Examples of such include, but are not limited to, a neomycin phosphotransferase (nptII) gene (Potrykus et al., 1985), which confers kanamycin resistance. Cells expressing the nptII gene can be selected using an appropriate antibiotic such as kanamycin or G418. Other commonly used selectable markers include the bar gene, which confers bialaphos resistance; a mutant EPSP synthase gene (Hinchee et al., 1988), which confers glyphosate resistance; a nitrilase gene, which confers resistance to bromoxynil (Stalker et al., 1988); a mutant acetolactate synthase gene (ALS), which confers imidazolinone or sulphonylurea resistance (European Patent Application 154,204, 1985); and a methotrexate resistant DHFR gene (Thillet et al., 1988).

Detail Description Paragraph - DETX (88):
[0115] This increase in sucrose export by fda-expressing leaves is an illustration of an increase in source capacity, very likely due to an increased carbon flow through the Calvin Cycle (in response to increased triose-P utilization) and thus an increase in net carbon utilization by the leaf. As seen in Table 2, the increase in sucrose loading in the phloem correlates with the level of fda expression.

Detail Description Paragraph - DETX (95):
[0121] For the cytosolic expression of the fda gene in corn plants, a construct was made in which the fda gene sequence was fused to the backbone of a vector containing the enhanced CaMV 35S promoter (e35S; Kay et al., 1987), the HSP70 intron (U.S. Pat. No. 5,593,874), and the NOS3' polyadenylation sequence (Fraley et al., 1983). This created a NotI cassette [P-e35S/HSP70 intron/fda/NOS3'] that was cloned into the NotI site of pMON30460, a monocot transformation vector, to form the plant transformation vector pMON13925, as shown in FIG. 5. pMON30460 contains an expression cassette for the selectable marker neomycin phosphotransferase typeII gene (nptII) [P-35S/NPTII/NOS3'] and a unique NotI site for cloning the gene of interest. The final vector (pMON13925) was constructed so that the gene of interest and the selectable marker gene were cloned in the same orientation. A vector fragment containing the expression cassettes for these gene sequences could be excised from the bacterial selector (Kan) and ori, gel purified, and used for plant transformation.

Detail Description Paragraph - DETX (99):
[0125] Transgenic maize plants transformed with the vectors pMON13925 (described above) or pMON17590 (described above) were produced using microprojectile bombardment, a procedure well-known to the art (Fromm, 1990; Gordon-Kamm et al., 1990; Walters et al., 1992). Embryogenic callus initiated from immature maize embryos was used as a target tissue. Plasmid DNA at 1 mg/mL in TE buffer was precipitated onto M10 tungsten particles using a calcium chloride/spermidine procedure, essentially as described by Klein et al. (1988). In addition to the gene of interest, the plasmids also contained the neomycin phosphotransferase II gene (nptII) driven by the 35S promoter from Cauliflower Mosaic Virus. The embryogenic callus target tissue was pretreated on culture medium osmotically buffered with 0.2M mannitol plus 0.2M sorbitol for approximately four hours prior to bombardment (Vain et al., 1993). Tissue was bombarded two times with the DNA-coated tungsten particles using the gunpowder version of the BioRad Particle Delivery System (PDS) 1000 device. Approximately 16 hours following bombardment, the tissue was subcultured onto a medium of the same composition except that it contained no mannitol or

sorbitol, and it contained an appropriate aminoglycoside antibiotic, such as G418", to select for those cells that contained and expressed the 35S/nptII gene. Actively growing tissue sectors were transferred to fresh selective medium approximately every 3 weeks. About 3 months after bombardment, plants were regenerated from surviving embryogenic callus essentially as described by Duncan and Widholm (1988).

Detail Description Paragraph - DETX (114):

[0139] A second potato transformation vector was constructed by cloning the NotI cassette [P-FMV/CTP2/fda/NOS3'] (described earlier) into the unique NotI site of pMON23616. pMON23616 is a potato transformation vector containing the nopaline-type T-DNA right border region (Fraley et al., 1985), an expression cassette for the neomycin phosphotransferase typeII gene [P-35S/NPTII/NOS3'] (selectable marker), a unique NotI site for cloning the gene expression cassette of interest, and the T-DNA left border region (Barker et al., 1983). Cloning of the NotI cassette [P-FMV/CTP2/fda/NOS3'] (described earlier) into the NotI site of pMON23616 results in the potato transformation vector pMON17581, as shown in FIG. 8. The vector pMON17581 was constructed such that the gene of interest and the selectable marker gene were transcribed in the same direction.

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TITLE: Methods and compositions for modifying oil and protein content in plants

PUBLICATION-DATE: January 29, 2004

INVENTOR-INFORMATION:

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Li, Changjiang	Urbandale	IA	US	

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US-CL-CURRENT: 800/281, 435/194 , 435/320.1 , 435/419 , 435/69.1 , 536/23.2

ABSTRACT:

Compositions and methods for altering pyruvate synthesis in plants are provided. The compositions and methods find use in increasing the oil or protein content of plant tissues, particularly seeds. Novel isolated nucleotide molecules encoding cytosolic and plastidic pyruvate kinases are provided. The methods involve introducing into a plant nucleotide sequences encoding enzymes that alter pyruvate synthesis. Additionally provided are isolated pyruvate kinases and transformed plants, plant tissues, plant cells, and seeds thereof.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 60/226,142, filed Aug. 18, 2000, which is hereby incorporated herein in its entirety by reference.

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Summary of Invention Paragraph - BSTX (24):
[0021] If desired, one or more additional nucleotide constructs may be introduced into the plant. Such additional nucleotide constructs can comprise, for example, at least a portion of a nucleotide sequence of an enzyme, or other protein, that is capable of affecting the synthesis of pyruvate in the cytosol. If expression of the nucleotide sequence is desired, the nucleotide construct can additionally comprise an operably linked promoter. The invention does not depend on a particular nucleotide sequence. Any nucleotide sequence originating from a gene or transcript that encodes an enzyme or protein that is capable of increasing or decreasing the synthesis and/or level of pyruvate in the cytosol can be employed. Typically, such an enzyme or protein is capable of altering the amount of **PEP that is available** in the cytosol for the

formation of pyruvate. If a decreased level or activity of such an enzyme or protein is desired, the methods of the invention may additionally comprise anti-sense suppression, co-suppression, or chimeraplasty.

Summary of Invention Paragraph - BSTX (29):
[0026] Methods are provided for altering the metabolism of pyruvate in plastids. The methods find use in increasing the synthesis of fatty acids and/or oil in a plant, or part thereof. In particular, the methods find use in the production of improved cultivars of crop plants having seeds with increased levels of oil. By increasing the flux of carbon toward oil biosynthesis, increased levels of oil, fatty acids, oil precursors, and specialty molecules that are synthesized from oil precursors can be achieved in a plant, or part thereof. The methods involve introducing into a plant at least one nucleotide construct comprising at least a portion of a nucleotide sequence encoding a pyruvate kinase or an NADP.sup.+-dependent malic enzyme. In some embodiments of the invention, both a pyruvate kinase nucleotide sequence and a NADP.sup.+-dependent malic enzyme nucleotide sequence are introduced into the plant. Such nucleotide sequences can be introduced into the plant as a single nucleotide molecule comprising both the pyruvate kinase and a NADP.sup.+-dependent malic enzyme nucleotide sequences. Alternatively, the pyruvate kinase and a NADP.sup.+-dependent malic enzyme nucleotide sequences can be introduced into the plant on two separate nucleotide molecules.

Summary of Invention Paragraph - BSTX (33):
[0030] If desired, one or more additional nucleotide constructs may be introduced into the plant. Such additional nucleotide constructs can comprise, for example, at least a portion of a nucleotide sequence encoding an enzyme, or other protein, that is capable of affecting the synthesis or the level of pyruvate in the plastid. If expression of the nucleotide sequence is desired, the nucleotide construct can additionally comprise an operably linked promoter. The invention does not depend on a particular nucleotide sequence. Any nucleotide sequence that encodes an enzyme, or protein, that is capable of increasing or decreasing the synthesis of pyruvate in the cytosol can be employed. Typically, such an enzyme or protein is capable of altering the amount of PEP, malate, or both that is available for the formation of pyruvate within the plastid. Such an enzyme can be localized in any compartment or organelle of the cell, including, but not limited to, the cytosol, plastids, mitochondria, and vacuoles. If a decreased level or activity of such an enzyme or protein is desired, the methods of the invention may additionally comprise anti-sense suppression, co-suppression, chimeraplasty, and dominant-negative strategies.

Summary of Invention Paragraph - BSTX (38):
[0035] To reduce or eliminate the conversion of malate to pyruvate in mitochondria, the plant can be transformed with a nucleotide construct comprising at least a portion of an NAD.sup.+-dependent malic enzyme nucleotide sequence. Malate that is formed in the cytosol, instead of entering the choloroplast, can enter the mitochondria where it can be decarboxylated to form pryuvate, which can then be converted into acetyl-CoA. Acetyl-CoA can then enter the TCA cycle. Reducing or eliminating the decarboxylation of malate in plant mitochondria by antisense suppression of mitochondrial NAD.sup.+-dependent malic enzyme is known to increase the flux of carbon to plastids in plant storage tissues. See WO 98/23757, herein incorporated by reference.

Summary of Invention Paragraph - BSTX (40):
[0037] While the methods of the present invention do not depend on a particular biological mechanism for increasing the oil or other desired product, it is recognized the methods of the present invention can lead to a

disruption in the metabolism of the plant leading to an increased flux of carbon flux to the plastids. This increased carbon flux may be the result of an increased rate of transport of malate from the cytosol to the plastids. Other cytosolic metabolites may, however, contribute to the increased flux of carbon to the plastid. Such metabolites include, but are not limited to, 3-phosphoglyceric acid and dihydroxyacetone phosphate.

Summary of Invention Paragraph - BSTX (89):

[0086] Root-preferred promoters are known and can be selected from the many available from the literature or isolated de novo from various compatible species. See, for example, Hire et al. (1992) Plant Mol. Biol. 20(2): 207-218 (soybean root-preferred glutamine synthetase gene); Keller and Baumgartner (1991) Plant Cell 3(10):1051-1061 (root-preferred control element in the GRP 1.8 gene of French bean); Sanger et al. (1990) Plant Mol. Biol. 14(3):433-443 (root-preferred promoter of the mannopine synthase (MAS) gene of Agrobacterium tumefaciens); and Miao et al. (1991) Plant Cell 3(1):11-22 (full-length cDNA clone encoding cytosolic glutamine synthetase (GS), which is expressed in roots and root nodules of soybean). See also Bogusz et al. (1990) Plant Cell 2(7):633-641, where two root-preferred promoters isolated from hemoglobin genes from the nitrogen-fixing nonlegume Parasponia andersonii and the related non-nitrogen-fixing nonlegume Trema tomentosa are described. The promoters of these genes were linked to a .beta.-glucuronidase reporter gene and introduced into both the nonlegume Nicotiana tabacum and the legume Lotus corniculatus, and in both instances root-preferred promoter activity was preserved. Leach and Aoyagi (1991) describe their analysis of the promoters of the highly expressed rolC and rolD root-inducing genes of Agrobacterium rhizogenes (see Plant Science (Limerick) 79(1):69-76). They concluded that enhancer and tissue-preferred DNA determinants are dissociated in those promoters. Teeri et al. (1989) used gene fusion to lacZ to show that the Agrobacterium T-DNA gene encoding octopine synthase is especially active in the epidermis of the root tip and that the TR2' gene is root specific in the intact plant and stimulated by wounding in leaf tissue, an especially desirable combination of characteristics for use with an insecticidal or larvicidal gene (see EMBO J. 8(2):343-350). The TR1' gene, fused to nptII (neomycin phosphotransferase II) showed similar characteristics. Additional root-preferred promoters include the VfENOD-GRP3 gene promoter (Kuster et al. (1995) Plant Mol. Biol. 29(4):759-772); and rolB promoter (Capana et al. (1994) Plant Mol. Biol. 25(4):681-691. See also U.S. Pat. Nos. 5,837,876; 5,750,386; 5,633,363; 5,459,252; 5,401,836; 5,110,732; and 5,023,179.

Summary of Invention Paragraph - BSTX (91):

[0088] Generally, the expression cassette will comprise a selectable marker gene for the selection of transformed cells. Selectable marker genes are utilized for the selection of transformed cells or tissues. Marker genes include genes encoding antibiotic resistance, such as those encoding neomycin phosphotransferase II (NEO) and hygromycin phosphotransferase (HPT), as well as genes conferring resistance to herbicidal compounds, such as glufosinate ammonium, bromoxynil, imidazolinones, and 2,4-dichlorophenoxyacetate (2,4-D). See generally, Yarranton (1992) Curr. Opin. Biotech. 3:506-511; Christopherson et al. (1992) Proc. Natl. Acad. Sci. USA 89:6314-6318; Yao et al. (1992) Cell 71:63-72; Reznikoff (1992) Mol. Microbiol. 6:2419-2422; Barkley et al. (1980) in The Operon, pp. 177-220; Hu et al. (1987) Cell 48:555-566; Brown et al. (1987) Cell 49:603-612; Figge et al. (1988) Cell 52:713-722; Deuschle et al. (1989) Proc. Natl. Acad. Aci. USA 86:5400-5404; Fuerst et al. (1989) Proc. Natl. Acad. Sci. USA 86:2549-2553; Deuschle et al. (1990) Science 248:480-483; Gossen (1993) Ph.D. Thesis, University of Heidelberg; Reines et al. (1993) Proc. Natl. Acad. Sci USA 90:1917-1921; Labow et al. (1990) Mol. Cell. Biol. 10:3343-3356; Zambretti et al. (1992) Proc. Natl. Acad. Sci. USA 89:3952-3956; Baim et al (1991) Proc. Natl.

Acad. Sci. USA 88:5072-5076; Wyborski et al. (1991) Nucleic Acids Res. 19:4647-4653; Hillenand-Wissman (1989) Topics Mol. Struc. Biol. 10:143-162; Degenkolb et al. (1991) Antimicrob. Agents Chemother. 35:1591-1595; Kleinschnidt et al. (1988) Biochemistry 27:1094-1104; Bonin (1993) Ph.D. Thesis, University of Heidelberg; Gossen et al. (1992) Proc. Natl. Acad Sci USA 89:5547-5551; Oliva et al. (1992) Antimicrob. Agents Chemother. 36:913-919; Hlavka et al. (1985) Handbook of Experimental Pharmacology, Vol. 78 (Springer-Verlag, Berlin); Gill et al. (1988) Nature 334:721-724. Such disclosures are herein incorporated by reference.

Detail Description Paragraph - DETX (28):

[0124] A selectable marker gene that can be used to facilitate soybean transformation is a transgene composed of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) Nature 313:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from E. coli; Gritz et al. (1983) Gene 25:179-188), and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of Agrobacterium tumefaciens. The expression cassette comprising the pyruvate kinase or malic enzyme nucleotide sequence operably linked to a seed-preferred promoter can be isolated as a restriction fragment. This fragment can then be inserted into a unique restriction site of the vector carrying the marker gene.

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TITLE: Nucleotide sequence of the haemophilus influenza Rd genome, fragments thereof, and uses thereof

PUBLICATION-DATE: January 29, 2004

INVENTOR-INFORMATION:

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child 08487429 19950607 US

parent continuation-in-part-of 08426787 19950421 US ABANDONED

US-CL-CURRENT: 435/6, 435/252.3 , 435/320.1 , 435/69.3 , 530/350 , 530/388.4 , 536/23.7

ABSTRACT:

The present invention provides the sequencing of the entire genome of Haemophilus influenzae Rd, SEQ ID NO: 1. The present invention further provides the sequence information stored on computer readable media, and computer-based systems and methods which facilitate its use. In addition to the entire genomic sequence, the present invention identifies over 1700 protein encoding fragments of the genome and identifies, by position relative to a unique NotI restriction endonuclease site, any regulatory elements which modulate the expression of the protein encoding fragments of the Haemophilus genome.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional of and claims priority under 35 U.S.C. .sctn. 120 to U.S. application Ser. No. 09/643,990, filed Aug. 23, 2000, which is a continuation of and claims priority under 35 U.S.C. .sctn. 120 to U.S. application Ser. No. 08/487,429, filed Jun. 7, 1995, which is a continuation-in-part of and claims priority under 35 U.S.C. .sctn. 120 to U.S. application Ser. No. 08/426,787, filed Apr. 21, 1995, which is hereby incorporated by reference in its entirety.

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Detail Description Paragraph - DETX (206):

[0242] In order to survive as a free living organism, H. influenaze must produce energy in the form of ATP via fermentation and/or electron transport. As a facultative anaerobe, H. influenaze Rd is known to ferment glucose, fructose, galactose, ribose, xylose and fucose (Dorocicz et al., J. Bacteriol. 175:7142 (1993)). The genes identified in Table 1(a) indicate that transport systems are available for the uptake of these sugars via the phosphoenolpyruvate-phosphotransferase system (PTS), and via non-PTS mechanisms. Genes that specify the common phosphate-carriers Enzyme I and Hpr (ptsI and ptsH) of the PTS system were identified as well as the glucose specific crr gene. The ptsH, ptsI, and crr genes constitute the pts operon. We have not however identified the gene encoding membrane-bound glucose specific Enzyme II. The latter enzyme is required for transport of glucose by the PTS system. A complete PTS system for fructose was identified.

Detail Description Table CWU - DETL (10):

HI1275 1347862 1348650 ferric enterobactin transport ATP-binding protein (fepC) [Escherichia coli] 29.4 51.3 238 HI1475 1555193 1554435 ferric enterobactin transport ATP-binding protein (fepC) [Escherichia coli] 33.2 54.8 220 HI1471 1549654 1551853 ferrichrome-iron receptor (fhuA) [Escherichia coli] 26.4 48.9 710 HI1388 1479930 1480475 ferritin like protein (rsgA) [Escherichia coli] 57.4 79.0 162 HI1389 1480494 1480988 ferritin like protein (rsgA) [Escherichia coli] 57.3 73.8 164 HI0363 385804 384887 iron(III) dicitrate transport ATP-binding protein FECE [Escherichia coli] 35.9 56.4 220 HI1274 1347324 1347861 iron(III) dicitrate transport system permease protein (fecD) [Escherichia 36.0 64.0 255 coli] HI1037 1099321 1100265 magnesium and cobalt transport protein (corA) [Escherichia coli] 70.3 84.8 316 HI0097 103798 104679 major ferric iron binding protein precursor (fbp) [Neisseria gonorrhoeae] 69.7 82.3 293 HI1051 1114308 1114635 mercuric transport protein (merT) [Pseudomonas aeruginosa] 25.0 55.2 99 HI1052 1114651 1114926 mercury scavenger protein (merP) [Pseudomonas fluorescens] 29.3 45.7 91 HI0294 327396 327193 mercury scavenger protein (merP) [Pseudomonas fluorescens] 32.8 67.2 67 HI1531 1594953 1594219 molybdate-binding periplasmic protein precursor (modB) [Azotobacter 21.7 43.0 245 vinelandii] HI0226 254880 253681 NA(+)/H(+) antiporter 1 (rhaA) [Escherichia coli] 52.6 74.6 380 HI0429 448992 450557 Na+/H+ antiporter (nhaB) [Escherichia coli] 70.6 87.5 501 HI1110 1171933 1170530 Na+/H+ antiporter (nhaC) [Bacillus firmus] 37.5 62.0 382 HI0098 104899 106317 periplasmic-binding-protein-dependent iron transport protein (stuB) 38.1 59.5 457 [Serratia marcescens] HI1479 1558763 1558167 periplasmic-binding-protein-dependent iron transport protein (sfuC) 39.9 58.0 197 [Serratia marcescens] HI0913 964424 966276 potassium efflux system (kelC) [Escherichia coli] 40.9 65.7 594 HI0292 326934 324769 potassium/copper-transportING ATPase A (copA) [Enterococcus faecalis] 42.9 64.4 723 HI1355 1429787 1428276 sodium/proline symporter (proline permease) (putP) [Escherichia coli] 62.8 79.1 489 HI0252 283326 282517 tonB protein (tonB) [Haemophilus influenzae] 96.2 98.5 261 HI0627 664922 666362 TRK system potassium uptake protein (trkA) [Escherichia coli] 65.8 83.4 458 Carbohydrates, organic alcohols & acids HI0020 22097 20661

2-oxoglutarate/malate translocator (SODiT1) [Spinacia oleracea] 35.8 59.6 452 HI0824 872894 873940 D-galactose-binding periplasmic protein (mglB) [Escherichia coli] 67.6 81.2 329 HI1113 1176024 1174516 D-xylose transport ATP-binding protein (xylG) [Escherichia coli] 71.5 85.8 501 HI1114 1177073 1176078 D-xylose-binding penplasmic protein (rbsB) [Escherichia coli] 76.0 88.4 328 HI1718 1785024 1783300 enzyme I (ptsI) [Salmonella typhimurium] 70.2 84.3 574 HI0182 194818 193967 formate transporter (formate channel) [Escherichia coli] 53.2 73.4 263 HI0450 471781 470285 fructose-permease IIA/FPR component (fruB) [Escherichia coli] 51.5 68.3 374 HI0448 469337 467670 fructose-permease IIBC component (truA) [Escherichia coli] 57.2 72.2 552 HI0614 643282 642851 fucose operon protein (fucu) [Escherichia coli] 66.3 80.0 94 HI0692 733673 734484 glpF protein (glpF) [Escherichia coli] 73.6 87.2 258 HI1019 1080518 1081194 glpF protein (glpP) [Escherichia coli] 30.6 54.6 208 HI1017 1078404 1079867 gluconate permease (gntP) [Bacillus subtilis] 29.1 56.4 442 HI1717 1783237 1782740 glucose phosphotransferase enzyme III-glc (crr) [Escherichia coli] 73.2 83.3 169 HI0688 729474 730914 glycerol-3-phosphatase transporter (glpT) [Escherichia coli] 64.5 78.9 445 HI0504 517869 519347 high affinity ribose transport protein (rbsA) [Escherichia coli] 71.1 85.4 494 HI0505 519363 520331 high affinity ribose transport protein (rbsC) [Escherichia coli] 68.0 86.5 303 HI0503 517436 517852 high affinity ribose transport protein (rbsD) [Escherichia coli] 59.0 78.4 139 HI0612 542139 640856 L-fucose permease (fucP) [Escherichia coli] 35.6 57.9 413 HI1221 1288578 1286983 L-lactate permease (lctP) [Escherichia coli] 30.2 53.9 532 HI1735 1802527 1801757 lactam utilization protein (lamB) [Emericella nidulans] 41.3 60.3 130 HI0825 874009 875526 mglA protein (mglA) [Escherichia coli] 73.9 84.6 506 HI0826 875546 876553 mglC protein (mglC) [Escherichia coli] 79.2 90.2 336 HI0506 520354 521229 periplasmic ribose-binding protein (rbsB) [Escherichia coli] 73.9 86.6 291 HI1719 1785361 1785107 phosphohistidinoprotein- -hexose phosphotransterase (ptsH) [Escherichia 77.6 88.2 85 coli] HI0830 878480 878773 potassium channel homolog (kch) [Escherichia coli] 67.7 80.2 96 HI0154 170140 168807 putative aspartate transport protein (dcuA) [Escherichia coli] 46.4 69.9 436 HI0748 803856 805175 putative aspartate transport protein (dcuA) [Escherichia coli] 42.6 70.1 435 HI1112 1174509 1173385 ribose transport permease protein (xylH) [Escherichia coli] 69.8 84.1 371 HI1696 1759373 1760743 sodium- and chloride-dependent GABA transporter [Homo sapiens] 29.3 52.6 471 HI0738 790926 789403 sodium-dependent noradrenaline transporter [Homo sapiens] 31.1 54.2 523 Nucleosides, purines & pyrimidines HI1089 1151815 1151024 ribonucleotide transport ATP-binding protein (mkl) [Mycobacterrum leprae] 42.2 61.5 244 HI1230 1296319 1295078 uracil permease (ursA) [Escherichia coli] 37.2 61.6 400 Anions HI1104 1164213 1165028 cysteine synthetase (cysZ) [Escherichia coli] 53.7 76.3 190 HI1697 1761825 1760773 hydrophilic membrane-bound protein (modC) [Escherichia coli] 55.9 74.5 263 HI1698 1762501 1761815 hydrophobic membrane-bound protein (modB) [Escherichia coli] 65.9 84.8 223 HI1384 1477430 1476585 integral membrane protein (patA) [Escherichia coli] 59.6 77.6 272 HI0356 380045 380764 nitrate transporter ATPase component (nasD) [Klebsiella pneumoniae] 34.9 57.8 254 HI1383 1475710 1475584 peripheral membrane protein B (pstB) [Escherichia coli] 77.0 86.8 256 HI1385 1478379 1477435 peripheral membrane protein C (pstC) [Escherichia coli] 57.3 78.7 300 HI1386 1479246 1478473 periplasmic phosphate-binding protein (pstS) [Escherichia coli] 49.8 67.7 256 HI1387 1479247 1479929 periplasmic phosphate-binding protein (pstS) [Escherichia coli] 63.8 75.4 69 HI1610 1669474 1670733 phosphate permease (YBR296C) [Saccharomyces cerevisiae] 35.6 60.0 551 Other HI0060 62564 60804 ATP dependent translocator homolog (msbA) [Haemophilus influenzae] 100.0

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DOCUMENT-IDENTIFIER: US 20040014118 A1

TITLE: Detection of group a streptococcus

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INVENTOR-INFORMATION:

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child 10465205 A1 20030619

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parent-patent 6593093 US

US-CL-CURRENT: 435/6, 435/91.2

ABSTRACT:

The invention provides methods to detect Group A Streptococcus (GAS) in biological samples using real-time PCR. Primers and probes for the detection of GAS are provided by the invention. Articles of manufacture containing such primers and probes for detecting GAS are further provided by the invention.

---------- KWIC ---------

Detail Description Paragraph - DETX (7):

[0021] A metabolic pathway chart showing the phosphoenolpyruvate:phosphotransferase system is available at http://www.genome.ad.jp/kegg/pathway/eco/-eco02060.html. Briefly, the phosphoenolpyruvate:phosphotransferase system (pep:pts or pts) is composed of two enzymes, HPr and enzyme I (or EI) encoded by the ptsH and ptsI genes, respectively. Enzyme I is autophosphorylated by phosphoenolpyruvate. Phosphorylated EI then catalyzes the phosphorylation of HPr in the membrane. HPr phosphorylates a sugar-specific enzyme that is translocated across the membrane. Thus, E1 and HPr are necessary for sugar translocation. The phosphotransferase system is reviewed by, for example, Postma et al. (1993, Microbiol. Rev., 57:543-94) and the pts operon is reviewed by, for example, Vadeboncoeur et al. (2000, J. Mol. Microbiol. Biotechnol., 2:483-90).

Detail Description Paragraph - DETX (8):

[0022] The invention provides methods to detect GAS by amplifying, for example, GAS nucleic acid molecules corresponding to a portion of the ptsI gene encoding enzyme I (EI) of the phosphoenolpyruvate:sugar phosphotransferase system. GAS nucleic acid molecules other than those exemplified herein (e.g.,

other than ptsI) also can be used to detect GAS in a sample and are known to those of skill in the art. Nucleic acid sequences encoding GAS ptsI have been described (see, for example, Ferretti et al., 2001, Proc. Natl. Acad. Sci. USA, 98:4658-63; and GenBank Accession Nos. NC 002737, and AE004092). Specifically, primers and probes to amplify and detect GAS ptsI nucleic acid molecules are provided by the invention.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040009466 A1

TITLE: Method for determining gene knockouts

PUBLICATION-DATE: January 15, 2004

INVENTOR-INFORMATION:

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APPL-NO: 10/ 616659

DATE FILED: July 9, 2003

RELATED-US-APPL-DATA:

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non-provisional-of-provisional 60444933 20030203 US

US-CL-CURRENT: 435/4, 435/6 , 702/20

ABSTRACT:

A method for determining candidates for gene deletions and additions using a model of a metabolic network associated with an organism, the model includes a plurality of metabolic reactions defining metabolite relationships, the method includes selecting a bioengineering objective for the organism, selecting at least one cellular objective, forming an optimization problem that couples the at least one cellular objective with the bioengineering objective, and solving the optimization problem to yield at least one candidate.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is based on U.S. Patent Application Serial No. 60/395,763, filed Jul. 10, 2002; U.S. Patent Application Serial No. 60/417,511, filed Oct. 9, 2002, and U.S. Patent Application Serial No. 60/444,933, filed Feb. 3, 2003, each of which is herein incorporated by reference in its entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX (12):

[0029] is a minimum level of biomass production. The vector v includes both internal and transport reactions. The forward (i.e., positive) direction of transport fluxes corresponds to the uptake of a particular metabolite, whereas the reverse (i.e., negative) direction corresponds to metabolite secretion. The uptake of glucose through the **phosphotransferase** system and glucokinase are denoted by v.sub.pts and v.sub.glk, respectively. Transport fluxes for

metabolites that can only be secreted from the network are members of .sub.secr.sub..sub.--.sub.onl- y. Note also that the complete set of reactions is subdivided into reversible .sub.rev and irreversible .sub.irrev reactions. The cellular objective is often assumed to be a drain of biosynthetic precursors in the ratios required for biomass formation (Neidhardt and Curtiss, 1996). The fluxes are reported per 1 gDW.multidot.hr such that biomass formation is expressed as g biomass produced/gDW.multidot.hr or 1/hr.

Detail Description Paragraph - DETX (31):

[0046] Which reactions, if any, that could be removed from the E. coli K-12 stoichiometric model (Edwards and Palsson, 2000) so as the remaining network produces succinate or lactate whenever biomass maximization is a good descriptor of flux allocation were identified. A prespecified amount of glucose (10 mmol/gDW.multidot.hr), along with unconstrained uptake routes for inorganic phosphate, oxygen, sulfate, and ammonia are provided to fuel the metabolic network. The optimization step could opt for or against the phosphotransferase system, glucokinase, or both mechanisms for the uptake of glucose. Secretion routes for acetate, carbon dioxide, ethanol, formate, lactate and succinate are also enabled. Note that because the glucose uptake rate is fixed, the biomass and product yields are essentially equivalent to the rates of biomass and product production, respectively. In all cases, the OptKnock procedure eliminated the oxygen uptake reaction pointing at anaerobic growth conditions consistent with current succinate (Zeikus et al., 1999) and lactate (Datta et al., 1995) fermentative production strategies.

Detail Description Paragraph - DETX (33):

[0048] A less intuitive strategy was identified for mutant C which focused on inactivating two PEP consuming reactions rather than eliminating competing byproduct (i.e., ethanol, formate, and lactate) production mechanisms. First, the phosphotransferase system was disabled requiring the network to rely exclusively on glucokinase for the uptake of glucose. Next, pyruvate kinase was removed leaving PEP carboxykinase as the only central metabolic reaction capable of draining the significant amount of PEP supplied by glycolysis. This strategy, assuming that the maximum biomass yield could be attained, resulted in a succinate yield approaching 88% of the theoretical maximum. In addition, FIG. 3A revealed significant succinate production for every attainable biomass yield, while the maximum theoretical yield of succinate is the same as that for the wild-type strain.

Detail Description Table CWU - DETL (1):

1 Succinate max v.sub.biomass min .SIGMA.(v.sub.0 - v).sup.2 Biomass Succinate Succinate ID Knockouts Enzyme (1/hr) (mmol/hr) (mmol/hr) Wild "Complete network" 0.38 0.12 0 A 1 COA + PYR .fwdarw. ACCOA + FOR Pyruvate formate lyase 0.31 10.70 1.65 2 NADH + PYR LAC + NAD Lactate dehydrogenase B 1 COA + PYR .fwdarw. ACCOA + FOR Pyruvate formate lyase 0.31 10.70 4.79 2 NADH + PYR LAC + NAD Lactate dehydrogenase 3 ACCOA + 2NADH COA + ETH + 2NAD Acetaldehyde dehydrogenase C 1 ADP + PEP .fwdarw. ATP + PYR Pyruvate kinase 0.16 15.15 6.21 2 ACTP + ADP AC + ATP or Acetate kinase ACCOA + Pi ACTP + COA Phosphotransacetylase 3 GLC + PEP .fwdarw. G6P + PYR Phosphotransferase system Lactate max v.sub.biomass min .SIGMA.(v.sub.0 - v).sup.2 Biomass Lactate Lactate ID Knockouts Enzyme (1/hr) (mmol/hr) (mmol/hr) Wild "Complete network" 0.38 0 0 A 1 ACTP + ADP AC + ATP or Acetate kinase 0.28 10.46 5.58 ACCOA + Pi ACTP + COA Phosphotransacetylase 2 ACCOA + 2NADH COA + ETH + 2NAD Acetaldehyde dehydrogenase B 1 ACTP + ADP AC + ATP or Acetate kinase 0.13 18.00 0.19 ACCOA + Pi ACTP + COA Phosphotransacetylase 2 ATP + F6P .fwdarw. ATP + FDP or Phosphofructokinase FDP T3P1 + T3P2 Fructose-1,6-bisphosphatate aldolase C 1 ACTP + ADP AC + ATP or Acetate kinase 0.12 18.13 10.53 ACCOA + Pi ACTP + COA Phosphotransacetylase 2 ATP + F6P .fwdarw. ATP + FDP or Phosphofructokinase FDP T3P1 + T3P2 Fructose-1,6-bisphosphatate aldolase 3

ACCOA + 2NADH COA + ETH + 2NAD Acetaldehyde dehydrogenase 4 GLC + ATP .fwdarw. G6P + PEP Glucokinase 1,3-Propanediol max v.sub.biomass min .SIGMA.(v.sub.0 - v).sup.2 Biomass 1,3-PD 1,3-PD ID Knockouts Enzyme (1/hr) (mmol/hr) (mmol/hr) Wild "Complete network" 1.06 0 0 A 1 FDP .fwdarw. F6P + Pi or Fructose-1,6-bisphosphatase 0.21 9.66 8.66 FDP T3P1 + T3P2 Fructose-1,6-bisphosphate aldolase 2 13PDG + ADP 3PG + ATP or Phosphoglycerate kinase NAD + Pi + T3P1 13PDG + NADH Glyceraldehyde-3-phosphate dehydrogenase 3 GL + NAD GLAL + NADH Aldehyde dehydrogenase B 1 T3P1 T3P2 Triosphosphate isomerase 0.29 9.67 9.54 2 G6P + NADP D6PGL + NADPH or Glucose 6-phosphate-1-dehydrogenase D6PGL .fwdarw. D6PGC 6-Phosphogluconolactonase 3 DR5P .fwdarw. ACAL + T3P1 Deoxyribose-phosphate aldolase 4 GL + NAD GLAL + NADH Aldehyde dehydrogenase

Claims Text - CLTX (18):

17. The method of claim 1 wherein the bioengineering objective is overproduction of succinate and at least one candidate is for gene deletion and comprising genes coding for the enzymes pyruvate formate lyase, acetaldehyde dehydrogenase, pyruvate kinase, FOF1-ATPase, NADH dehydrogenase I, fumarase, D-Lactate dehydrogenase, pyridine nucleotide transhydrogenase, phosphotransacetylase, acetate kinase, **phosphotransferase,** or combinations thereof.

US-PAT-NO: 6846651

DOCUMENT-IDENTIFIER: US 6846651 B2

TITLE: Nucleotide sequence of the Haemophilus influenzae Rd genome, fragments thereof, and uses thereof

DATE-ISSUED: January 25, 2005

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Fleischmann; Robert D.	Gaithersburg	MD	N/A	N/A
Adams; Mark D.	Rockville	MD	N/A	N/A
White; Owen	Gaithersburg	MD	N/A	N/A
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APPL-NO: 10/ 158865

DATE FILED: June 3, 2002

PARENT-CASE:

This appln is a DIV of Ser. No. 09/557,884, filed Apr. 25, 2000, now U.S. Pat. No. 6,506,881 which is a con of Ser. No. 08/476,102 filed Jun. 7, 1995, now U.S. Pat. No. 6,355,450 which is a CIP of Ser. No. 08/426,787 filed Apr. 21, 1995, abandoned.

US-CL-CURRENT: 435/69.1, 435/252.3 , 435/320.1 , 536/23.7

ABSTRACT:

The present invention provides the sequencing of the entire genome of Haemophilus influenzae Rd, SEQ ID NO:1. The present invention further provides the sequence information stored on computer readable media, and computer-based systems and methods which facilitate its use. In addition to the entire genomic sequence, the present invention identifies over 1700 protein encoding fragments of the genome and identifies, by position relative to a unique Not I restriction endonuclease site, any regulatory elements which modulate the expression of the protein encoding fragments of the Haemophilus genome.

50 Claims, 47 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 47

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Detailed Description Text - DETX (203):

In order to survive as a free living organism, H. influenaze must produce energy in the form of ATP via fermentation and/or electron transport. As a facultative anaerobe, H. influenaze Rd is known to ferment glucose, fructose, galactose, ribose, xylose and fucose (Dorocicz et al., J. Bacteriol. 175:7142 (1993)). The genes identified in Table 1(a) indicate that transport systems are **available for the uptake of these sugars via the**

phosphoenolpyruvate-phosphotransferase system (PTS), and via non-PTS mechanisms. Genes that specify the common phosphatecarriers Enzyme I and Hpr (ptsI and ptsH) of the PTS system were identified as well as the glucose specific crr gene. The ptsH, ptsI, and crr genes constitute the pts operon. We have not however identified the gene encoding membrane-bound glucose specific Enzyme II. The latter enzyme is required for transport of glucose by the PTS system. A complete FTS system for fructose was identified.

Detailed Description Paragraph Table - DETL (8):

influenzae) 96.2 98.5 261 HI0627 664922 666362 TRK system potassium uptake protein (trkA) (Escherichia coli) 85.8 83.4 458 Carbohydrates, organic alcohols & acids HI0020 22097 20661 2-oxoglutarate/malate translocator (SOD/T1) (Spinacia cleraces) 35.8 59.6 452 HI0824 872894 873940 D-galactose-binding periplasmic protein (mglB) (Escherichia coli) 67.6 81.2 329 HI1113 1176024 1174516 D-xylose transport ATP-binding protein (xylG) (Escherichia coli) 71.5 85.8 501 HI1114 1177073 1176076 D-xylose-binding periplasmic protein (rbsB) (Escherichia coli) 76.0 88.4 328 HI1718 1785024 1783300 enzyme 1 (ptsI) (Salmonella typhimurium) 70.2 84.3 574 HI0182 194818 193967 formate transporter (formate channel) (Escherichia coli) 53.2 73.4 263 HI0450 471781 470285 fructose-permease IIA/FPR component (fruB) (Escherichia coli) 51.5 68.3 374 HI0448 469337 467670 fructose-permease IIBC component (fruA) (Escherichia coli) 57.2 72.2 552 HI0614 643282 642851 fucose operon protein (fucU) (Escherichia coli) 66.3 80.0 94 HI0692 733673 734464 glpF protein (glpF) (Escherichia coli) 73.6 87.2 258 HI1019 1080518 1081194 glpF protein (glpF) (Escherichia coli) 30.6 54.6 208 HI1017 1078404 1079867 gluconate permease (gntP) (Bacillus subtilis) 29.1 56.4 442 HI1717 1783237 1782740 glucose phosphotransferase enzyme III-glc (crr) (Escherichia coli) 73.2 83.3 169 HI0688 729474 730914 glycerol-3-phosphatase transporter (glpT) (Escherichia coli) 64.5 78.9 445 HI0504 517869 519347 high affinity ribose transport protein (rbsA) (Escherichia coli) 71.1 85.4 494 HI0505 519363 520331 high affinity ribose transport protein (rbsC) (Escherichia coli) 68.0 86.5 303 HI0503 517436 517852 high affinity ribose transport protein (rbsD) (Escherichia coli) 59.0 78.4 139 HI0612 642139 640856 L-fucose permease (fucP) (Escherichia coli) 35.6 57.9 413 HI1221 1288578 1286983 L-lactate permease (lctP) (Escherichia coli) 30.2 53.9 532 HI1735 1802527 1801757 lactam utilization protein (lamB) (Emericella ridulans) 41.3 60.3 130 HI0825 874009 875526 mglA protein (mglA) (Escherichia coli) 73.9 84.6 506 HI0826 875546 876553 mglC protein (mglC) (Escherichia coli) 79.2 90.2 336 HI0506 520354 521229 periplasmic ribose-binding protein (rbsB) (Escherichia coli) 73.9 86.6 291 HI1719 1785361 1785107 phosphohistidinoprotein-hexose phosphotransferase (ptsH) (Escherichia 77.6 88.2 85 coli) HI0830 878480 876773 potassium channel homolog (kch) (Escherichia coli) 67.7 80.2 96 HI0154 170140 168807 putative aspartate transport protein (dcuA) (Escherichia coli) 46.4 59.9 436 HI0748 803856 805175 putative aspartate transport protein (dcuA) (Escherichia coli) 42.6 70.1 435 HI1112 1174509 1173385 ribose transport permease protein (xylH) (Escherichia coli) 69.8 84.1 371 HI1696 1759373 1760743 sodium- and chloride-dependent GABA transporter (Homo sapiens) 29.3 52.6 471 HI0738 790926 789403 sodium-dependent noradrenaline transporter (Homo sapiens) 31.1 54.2 523 Nucleosides, purines & pyrimidines HI1089 1151815 1151024 ribonucleotide transport ATP-binding protein (mkl) (Mycrobacterium leprae) 42.2 61.5 244 HI1230 1296319 1295078 uracil permease (uraA) (Escherichia coli) 37.2 61.6 400 Anions HI1104 1164213 1165028 cysteine synthetase (cysZ) (Escherichia coli) 53.7 76.3 190 HI1697 1761825 1760773 hydrophilic membrane-bound protein (modC) (Escherichia coli) 55.9 74.5 263 HI1698 1762501 1761815 hydrophobic membrane-bound protein (modB) (Escherichia coli) 65.9 84.8 223 HI1384 1477430 1476585 integral membrane protein (pstA) (Escherichia coli) 59.6 77.6 272 HI0358 380045 380764 nitrate transporter ATPase component (nasD) (Klebsiella pneumoniae) 34.9 57.8 254 HI1383 1475710 1476584 peripheral membrane protein B (pstB) (Escherichia coli) 77.0 86.8 256 HI1385 1478379

1477435 peripheral membrane protein C (pstC) (Escherichia coli) 57.3 78.7 300 HI1386 1479246 1478473 periplasmic phosphate-binding protein (pstS) (Escherichia coli) 49.8 67.7 256 HI1387 1479247 1479929 periplasmic phosphate-binding protein (pstS) (Escherichia coli) 63.8 75.4 89 HI1610 1669474 1670733 phosphate permease (YBR296C) (Saccharomyces cerevisiae) 35.6 60.0 551 Other HI0060 62564 60804 ATP dependent translocator homolog (msbA) (Haemophilus influenzae) 100.0 100.0 458 HI0623 653683 662010 ATP-binding protein (abc) (Escherichia coli) 74.0 86.5 200 HI1625 1686470 1686186 cystic fibrosis transmembrane conductance regulator (Bos taurus) 35.3 60.8 233 HI0855 899042 900688 heme-binding lipoprotein (dppA) (Haemophilus influenzae) 98.9 99.3 547 HI0266 295639 298353 heme-hemopexin-binding protein (hxuA) (Haemophilus influenaze) 82.1 89.5 928 HI1476 1556199 1555189 hemin permease (hemU) (Yersinia enterocolitica) 36.1 62.7 325 HI0264 291684 293852 hemin receptor precursor (hemR) (Yersinia enterocolitica) 28.5 45.9 578 HI1712 1779487 1777481 high-affinity choline transport protein (betT) (Escherichia coli) 34.7 61.6 653 HI0663 705327 703054 lactoferrin binding protein (lbpA) (Neisseria meningitidis) 30.2 47.9 763 HI0610 637954 639336 Na+/sulfate cotransporter (Rattus norvegicus) 34.4 57.8 562 HI0977 1032420 1033871 pantothenate permease (panF) (Escherichia coli) 60.2 77.9 478 HI0714 760739 757488 transferrin binding protein 1 precursor (tbp1) (Neisseria meningitidis) 29.9 48.6 894 HI0996 1059604 1056869 transferrin binding protein 1 precursor (tbp1) (Neisseria meningitidis) 51.2 69.5 885 HI1220 1286725 1283987 transferrin binding protein 1 precursor (tbp1) (Neisseria meningtidis) 28.4 46.8 902 HI0997 1061509 1059635 transferrin binding protein 1 precursor (tbp1) (Neisseria meningtidis) 39.9 54.7 692 HI0975 1029676 1030542 transferrin-binding protein (ttbA) (Actinobacillus pleuropneumoniae) 28.9 48.0 578 HI1571 1633105 1633993 transferrin-binding protein 1 (tbp1) (Neisseria meningitidis) 41.3 59.5 727 HI0837 676956 674098 transferrin-binding protein 1 (tbp2) (Neisseria gonorrhoeae) 31.6 51.7 828 HI0665 706622 708309 transport ATP-binding protein (cydD) (Escherichia coli) 26.4 54.0 561 HI1160 1228897 1225140 transport ATP-binding protein (cydD) (Escherichia coli) 50.7 73.5 588 Cellular processes Chaperones HI0544 565037 565324 chaperonin (groES) (mopB) (Escherichia coli) 87.5 94.8 96 HI0545 565350 566993 heat shock protein (groEL) (mopA) (Haemophilus ducreyi) 89.8 94.9 547 HI1241 1310497 1311678 heat shock protein (dnaJ) (Escherichia coli) 68.0 82.5 376 HI0104 111572 109680 heat shock protein C62.5 (htpG) (Escherichia coli) 75.4 88.3 621 HI0375 396463 394607 hsc66 protein (hsc66) (Escherichia coli) 69.2 82.0 616 HI1240 1308539 1310443 hsp70 protein (dnaK) (Escherichia coli) 78.5 88.2 638 Cell division HI0771 831200 831853 cell division ATP-binding protein (tts) (Escherichia coli) 64.1 78.3 216 HI1211 1275245 1274358 cell division inhibitor (sulA) (Vibrio cholerae) 33.9 55.7 116 HI1145 1210058 1211332 cell division protein (itsA) (Escherichia coli) 52.8 74.2 420 HI1338 1410017 1412129 cell division protein (itsH) (Escherichia coli) 75.2 87.8 624 HI1470 1549516 1548374 cell division protein (itsH) (Escherichia coli) 77.8 88.3 369 HI1337 1409390 1410016 cell division protein (itsJ) (Escherichia coli) 81.7 90.4 208 HI1134 1196901 1197221 cell division protein (itsL) (Escherichia coli) 36.6 60.4 101 HI1144 1209275 1210036 cell division protein (itsQ) (Escherichia coli) 40.6 58.5 231 HI1140 1204467 1205648 cell division protein (itsW) (Escherichia coli) 52.3 74.9 374 HI0770 829937 831178 cell division protein (itsY) (Escherichia coli) 66.0 61.1 497 HI1146 1211419 1212681 cell division protein (itsZ) (Escherichia coli) 67.2 83.1 306 HI1377 1465224 1469760 cell division protein (mukB) (Escherichia coli) 61.4 77.3 1455 HI1356 1429903 1431375 cytoplasmic axial filament protein (cefA) (Escherichia coli) 71.0 66.3 488 HI0772 831866 832795 ltsX protein (ltsX) (Escherichia coli) 43.5 69.9 292 HI1067 1128511 1129221 mukB suppressor protein (smba) (Escherichia coli) 77.4 90.2 235 HI1135 1197237 1199067 penicillin-binding protein 3 (ftsI) (Escherichia coli) 52.8 70.7 564 Protein, peptide secretion HI0016 17278 15485 GTP-binding membrane protein (lepA) (Escherichia coli) 85.6 91.0 597 HI1472 1551915 1553681 colicin V secretion ATP-binding protein

(cvaB) (Escherichia coli) 29.9 56.0 373 HI1008 1070885 1071397 lipoprotein signal peptidase (lspA) (Escherichia coli) 51.3 71.5 158 HI1648 1706947 1707753 peptide transport system ATP-binding protein SAPF (sapF) (Escherichia coli) 49.6 70.8 264 HI0718 764525 764842 preprotein translocase (secE) (Escherichia coli) 40.6 62.3 106 HI0600 848438 849760 preprotein translocase SECY subunit (secY) (Escherichia coli) 74.7 86.9 443 HI0241 269734 267887 protein-export membrane protein (secD) (Escherichia coli) 59.6 77.3 615 HI0240 267876 266902 protein-export membrane protein (secF) (Escherichia coli) 48.0 73.0 302 HI0447 466800 467135 protein-export membrane protein (secG) (Escherichia coli) 58.9 81.3 110 HI0745 801965 801459 protein-export protein (secB) (Escherichia coli) 56.2 80.8 145 HI0911 961135 963837 secA protein (secA) (Escherichia coli) 88.0 81.7 896 HI0015 15473 14427 signal peptidase I (lepB) (Escherichia coli) 46.3 65.1 319 HI0106 114073 112688 signal recognition particle protein (54 homolog) (lfh) (Escherichia coli) 79.9 90.9 452 HI0715 761040 762335 trigger factor (tig) (Escherichia coli) 64.4 80.3 432 HI0298 330445 329758 type 4 prepilin-like protein specific leader peptidase (hopD) (Escherichia 27.2 49.0 208 coli) HI0299 331661 330445 xcpS protein (xcpS) (Pseudomonas putida) 29.2 56.7 398 Detoxification HI0930 985290 966813 KW20 catalase (hktE) (Haemophilus influenzae) 99.2 99.4 508 HI1090 1152892 1152248 superoxide dismutase (sodA) (Haemophilus influenzae) 99.0 99.5 209 HI1004 1065726 1067108 thiophene and furan oxidation protein (thdF) (Escherichia coli) 73.8 85.4 451 Cell killing HI0303 334801 335697 hemolysin (tlyC) (Serpulina hyodysenteriae) 36.9 57.5 252 HI1664 1723070 1723648 hemolysin, 21 kDa (hly) (Actinobacillus pleuropneumoniae) 54.5 72.4 156 HI1376 1464493 1465221 killing protein (kicA) (Escherichia coli) 69.0 83.6 222 HI1375 1463019 1464443 killing protein suppressor (kicB) (Escherichia coli) 66.9 83.0 440 HI1053 1116898 1115057 leukotoxin secretion ATP-binding protein (lktB) (Actinobacillus 34.2 55.1 512 actinomycetemcomitans) Transformation HI0436 456360 455674 com101A protein (comF) (Haemophilus influenzae) 100.0 100.0 229 HI1010 1072519 1072854 competence locus E (comE1) (Bacillus subtilis) 46.7 70.0 59 HI0603 622277 622927 tfoX protein (tfoX) (Haemophilus influenzae) 99.5 99.5 217 HI0443 462729 463571 transformation gene cluster hypothetical protein (GB:M62809_1) (com) 100.0 100.0 281 (Haemophilus influenzae) HI0435 455595 455002 transformation gene cluster hypothetical protein (GB:M62809_10) (com) 99.5 99.5 198 (Haemophilus influenzae) HI0442 460047 462638 transformation gene cluster hypothetical protein (GB:M62809_2) (com) 100.0 100.0 864 (Haemophilus influenzae) HI0441 459948 459154 transformation gene cluster hypothetical protein (GB:M62809_3) (com) 100.0 100.0 265 (Haemophilus influenzae)

US-PAT-NO: 6833490

DOCUMENT-IDENTIFIER: US 6833490 B1

TITLE: Regulating metabolism by modifying the level of trehalose-6-phosphate

DATE-ISSUED: December 21, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Goddijn; Oscar Johannes Maria	Leider		N/A	N/A	NL
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APPL-NO: 09/ 171937

DATE FILED: April 28, 1999

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
NL	96201225	May 3, 1996
NL	96202128	July 26, 1996
NL	96202395	August 29, 1996

PCT-DATA:
APPL-NO: PCT/EP97/02497
DATE-FILED: May 2, 1997
PUB-NO: WO97/42326
PUB-DATE: Nov 13, 1997
371-DATE: Apr 28, 1999
102(E)-DATE:Apr 28, 1999

US-CL-CURRENT: 800/284, 435/101 , 435/194 , 435/468 , 435/469 , 800/278 , 800/287 , 800/288 , 800/290 , 800/294

ABSTRACT:

The invention involves decreasing the intracellular availability of trehalose-6-phosphate by plant cell transformation with a gene encoding trehalose-6-phosphate phosphatase from E. coli. Phenotypic effects of plant transformation with this gene include stimulation of glycolysis, cell or tissue growth, and metabolism; and inhibition of photosynthesis and bolting.

20 Claims, 51 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 42

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Brief Summary Text - BSTX (13):
It has now been found that modification of the development and/or composition of cells, tissue and organs in vivo is possible by introducing the

enzyme trehalose-6-phosphate synthase (TPS) and/or trehalose-6-phosphatase phosphate (TPP) thereby inducing a change in metabolic pathways of the saccharide trehalose-6-phosphate (T-6-P) resulting in an alteration of the intracellular availability of T-6-P. Introduction of TPS thereby inducing an increase in the intracellular concentration of T-6-P causes inhibition of carbon flow in the glycolytic direction, stimulation of the photosynthesis, inhibition of growth, stimulation of sink-related activity and an increase in storage of resources. Introduction of TPP thereby introducing a decrease in the intracellular concentration of T-6-P causes stimulation of carbon flow in the glycolytic direction, increase in biomass and a decrease in photosynthetic activity.

Drawing Description Text - DRTX (2):

FIG. 1. Schematic representation of plasmid pVDH275 harbouring the neomycin-phosphotransferase gene (NPTII) flanked by the 35S cauliflower mosaic virus promoter (P35S) and terminator (T35S) as a selectable marker; an expression cassette comprising the pea plastocyanin promoter (pPCpea) and the nopaline synthase terminator (Tnos); right (RB) and left (LB) T-DNA border sequences and a bacterial kanamycin resistance (KanR) marker gene.

Detailed Description Text - DETX (15):

Generation of the "plenty" signal by decreasing the intracellular concentration of trehalose-6-phosphate through expression of the enzyme TPP (or inhibition of the enzyme TPS) will signal all cell systems to increase glycolytic carbon flow and inhibit photosynthesis. This is nicely shown in the experimental part, where for instance in Experiment 2 transgenic tobacco plants are described in which the enzyme TPP is expressed having increased leaf size, increased branching and a reduction of the amount of chlorophyll. However, since the "plenty" signal is generated in the absence of sufficient supply of glucose, the pool of carbohydrates in the cell is rapidly depleted.

Detailed Description Text - DETX (50):

To select or screen for transformed cells, it is preferred to include a marker gene linked to the plant expressible gene according to the invention to be transferred to a plant cell. The choice of a suitable marker gene in plant transformation is well within the scope of the average skilled worker; some examples of routinely used marker genes are the neomycin phosphotransferase genes conferring resistance to kanamycin (EP-B 131 623), the glutathion-S-transferase gene from rat liver conferring resistance to glutathione derived herbicides (EP-A 256 223), glutamine synthetase conferring upon overexpression resistance to glutamine synthetase inhibitors such as phosphinothricin (WO 87/05327), the acetyl transferase gene from Streptomyces viridochromogenes conferring resistance to the selective agent phosphinothricin (EP-A 275 957), the gene encoding a 5-enolshikimate-3-phosphate synthase (EPSPS) conferring tolerance to N-phosphonomethylglycine, the bar gene conferring resistance against Bialaphos (e.g. WO 91/02071) and the like. The actual choice of the marker is not crucial as long as it is functional (i.e. selective) in combination with the plant cells of choice.

Other Reference Publication - OREF (26):

Hajirezaei, M. et al. "Transgenic potato plants with strongly decreased expression of pyrophosphate: fructose-6-phosphate phosphotransferase show no visible phenotype and only minor changes in metabolic fluxes in their tubers." Plant, vol. 192(1994),pp. 16-30.

US-PAT-NO: 6822142

DOCUMENT-IDENTIFIER: US 6822142 B2

TITLE: Transgenic plants containing altered levels of steroid compounds

DATE-ISSUED: November 23, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Karunanandaa; Balasulojini	St. Louis	MO	N/A	N/A
Post-Beittenmiller; Martha	St. Louis	MO	N/A	N/A
Venkatramesh; Mylavarapu	St. Louis	MO	N/A	N/A
Kishore; Ganesh M.	St. Louis	MO	N/A	N/A
Thorne; Gregory M.	St. Louis	MO	N/A	N/A
LeDeaux; John R.	St. Louis	MO	N/A	N/A

APPL-NO: 09/ 885723

DATE FILED: June 20, 2001

US-CL-CURRENT: 800/298, 435/320.1 , 435/419 , 536/23.6 , 800/278

ABSTRACT:

Disclosed are constructs comprising sequences encoding 3-hydroxy-3methylglutaryl-Coenzyme A reductase and at least one other sterol synthesis pathway enzyme. Also disclosed are methods for using such constructs to alter sterol production and content in cells, plants, seeds and storage organs of plants. Also provided are oils and compositions containing altered sterol levels produced by use of the disclosed constructs. Novel nucleotide sequences useful in the alteration of sterol production are also provided. Also provided are cells, plants, seeds and storage organs of plants comprising sequences encoding 3-hydroxy-3methylglutaryl-Coenzyme A reductase, at least one other sterol synthesis pathway enzyme and at least one tocopherol synthesis enzyme.

18 Claims, 78 Drawing figures

Exemplary Claim Number: 18

Number of Drawing Sheets: 78

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Drawing Description Text - DRTX (8):

FIG. 6 is a map showing the structure of construct pMON43818. pMON43818 is a recombinant binary vector carrying the gene encoding rubber hydroxymethyl glutaryl CoA reductase1 (HMGR1) in sense orientation driven by the soybean alpha' beta conglycinin promoter. P-NOS: nopaline synthase gene promoter; kan: coding region for neomycin **phospho transferase** protein to confer resistance to kanamycin; NOS 3': 3' termination end of nopaline synthase coding region; Soy Alpha' Beta Conglycinin: 7S alpha' beta conglycinin gene promoter from soybean; Rubber HMGR1 gene: coding sequence for HMGR1 gene from Hevea brasiliensis; E9 3': 3' end of pea rbcS E9 gene; Left border: octopine left border, sequence

essential for transfer of T-DNA into Agrobacterium; ori-V: plasmid origin of replication in Agrobacterium; rop: coding sequence for repressor of primer; Ori-322: origin of replication in E. coli; Spc/Str: coding region for Tn7 adenylyltransferase (AAD(3")) conferring resistance to spectinomycin and streptomycin; Right Border: right border sequence of T-DNA essential for integration into Agrobacterium.

Drawing Description Text - DRTX (9):

FIG. 7 is a map showing the structure of construct pMON43052. pMON43052 is a recombinant shuttle vector, carrying the cDNA fragment encoding the catalytic domain of Arabidopsis HMGR1 in sense orientation driven by the soybean alpha' beta conglycinin promoter. P-NOS: nopaline synthase gene promoter; kan: coding region for neomycin phosphotransferase protein to confer resistance to kanamycin; NOS 3': 3' termination end of nopaline synthase coding region; Soy Alpha' Beta Conglycinin: 7S alpha' beta conglycinin gene promoter from soybean; Arabidopsis HMGR catalytic domain: coding sequence for the catalytic domain of Arabidopsis HMGR1 protein; E9 3': 3' end of pea rbcS E9 gene; Left border: octopine left border, sequence essential for transfer of T-DNA into Agrobacterium; ori-V: plasmid origin of replication in Agrobacterium; rop: coding sequence for repressor of primer; Ori-322: origin of replication in E. coli; Spc/Str: coding region for Tn7 adenylyltransferase (AAD(3")) conferring resistance to spectinomycin and streptomycin; Right Border: right border sequence of T-DNA essential for integration into Agrobacterium.

Drawing Description Text - DRTX (10):

FIG. 8 is a map showing the structure of construct pMON51850. pMON51850 is a binary vector for Agrobacterium mediated transformation of soybean. P-NOS: nopaline synthase gene promoter; kan: coding region for neomycin phosphotransferase protein to confer resistance to kanamycin; NOS 3': 3' termination end of nopaline synthase coding region; Left border: octopine left border sequence essential for transfer of T-DNA into Agrobacterium; ori-v: plasmid origin of replication in Agrobacterium; rop: coding sequence for repressor of primer; ori-322: origin of replication in E. coli; Spc/Str: coding region for Tn7 adenylyltransferase (AAD(3")) conferring resistance to spectinomycin and streptomycin; Right Border: right border sequence of T-DNA essential for integration into Agrobacterium.

Drawing Description Text - DRTX (11):

FIG. 9 is a map showing the structure of construct pMON43057. pMON43057 is a recombinant binary vector for Agrobacterium mediated transformation of soybean, carrying the gene cassette for expressing catalytic domain of HMGR1 from Arabidopsis thaliana. The catalytic domain of the HMGR1 cDNA is driven by soybean 7S alpha' beta conglycinin promoter. P-NOS: nopaline synthase gene promoter; kan: coding region for neomycin phosphotransferase protein to confer resistance to kanamycin; NOS 3': 3' termination end of nopaline synthase coding region; Left border: octopine left border sequence essential for transfer of T-DNA into Agrobacterium; ori-V: plasmid origin of replication in Agrobacterium; rop: coding sequence for repressor of primer; ori-322: origin of replication in E. coli; Spc/Str: coding region for Tn7 adenylyltransferase (AAD(3")) conferring resistance to spectinomycin and streptomycin; Right Border: right border sequence essential for transfer of T-DNA into Agrobacterium; Soy Alpha' Beta Conglycinin: soybean 7S alpha' beta conglycinin gene promoter; Arabidopsis HMGR catalytic domain: coding sequence for Arabidopsis HMGR1 catalytic domain; E9 3': 3' end of pea rbcS E9 gene.

Drawing Description Text - DRTX (12):

FIG. 10 is a map showing the structure of construct pMON43058. pMON43058 is a recombinant binary vector for Agrobacterium-mediated soybean transformation, carrying gene expression cassettes for catalytic domain of HMGR1 from

Arabidopsis thaliana and SMTII from Arabidopsis thaliana. P-NOS: nopaline synthase gene promoter; kan: coding region for neomycin phosphotransferase protein to confer resistance to kanamycin; NOS 3': 3' termination end of nopaline synthase coding region; Left border: octopine left border sequence essential for transfer of T-DNA into Agrobacterium; ori-V: plasmid origin of replication in Agrobacterium; rop: coding sequence for repressor of primer; ori-322: origin of replication in E. coli; Spc/Str: coding region for Tn7 adenylyltransferase (AAD(3")) conferring resistance to spectinomycin and streptomycin; Right Border: right border sequence essential for transfer of T-DNA into Agrobacterium; Soy Alpha' Beta Conglycinin: 7S alpha' beta conglycinin gene promoter from soybean; Arabidopsis HMGR catalytic domain: sequence encoding the catalytic domain of Arabidopsis HMGR1; E9 3': 3' end of pea rbcS E9 gene; Soy Alpha' Beta Conglycinin: soybean 7S alpha' beta conglycinin gene promoter; Arabidopsis SMT2: cDNA encoding sterol methyl transferase II enzyme from Arabidopsis thaliana (accession no: X89867); NOS 3': 3' termination end of nopaline synthase coding region.

Detailed Description Text - DETX (207):

In preferred embodiments, the vector used to express the polypeptide coding gene includes a selection marker that is effective in a plant cell, preferably a drug resistance selection marker. One preferred drug resistance marker is the gene whose expression results in kanamycin resistance; i.e., the chimeric gene containing the nopaline synthase promoter, Tn5 neomycin phosphotransferase II and nopaline synthase 3' nontranslated region described by Rogers et al., in Methods for Plant Molecular Biology, A. Weissbach and H. Weissbach eds., Academic Press Inc., San Diego, Calif. (1988).

Detailed Description Text - DETX (342):

Total sterols increased by 3.2- and 3.9-fold in the best performing plants (transgenic events 3 and 4). These two events also showed the highest increases of individual sterols. Campesterol increased by 2.7-fold, sitosterol by 3.4-fold, sitostanol by 3.2-fold and other sterols by 6.5-fold in event 3 while stigmasterol increased by 2.3-fold in event 4. The other sterols, which account for the highest increase in total sterols were pathway intermediates that included squalene, cycloartenol, 24-methylene cycloartenol, obtusifoliol, isofucosterol, and stigmasta-7-enol. These pathway intermediates normally form minor constituents in the sterol composition of seeds. However, in the transgenic seeds, probably due to increased carbon flux through the pathway, they accumulate in significant amounts. This suggests additional control points for sterol biosynthesis in plants such as squalene epoxidase, C-24 sterol methyltransferase, and C-14 obtusifoliol demethylase.

Detailed Description Text - DETX (353):

Six transgenic lines haboring pMON43058 produced 5.8- to 6-fold increase in total sterols and the rest of the 10 transgenic lines with the pMON43058 showed 3- to 5-fold increase in total sterols. The best performing transgenic lines showed about 2- to 3-fold increase in sitosterol and 4.5- to 6-fold increase in sitostanol levels. However, the campesterol accumulation was reduced by 50% in these lines. This was due to overexpression of the Arabidopsis SMTII enzyme which enhances the carbon flux towards the synthesis of 24-ethyl sterols thereby reducing the carbon flux through the pathway leading to the synthesis of 24-methyl sterols. As seen in pMON43057 transgenic lines, all of the transgenic lines harboring the pMON43058 also accumulated 50-60% of the total sterols in the form of pathway intermediates which are squalene, cycloartenol, 24-methylene cycloartenol, obtusifoliol, isofucosterol, and stigmasta-7-enol. These pathway intermediates normally form minor constituents in the sterol composition of seeds. However, in the transgenic seeds, probably due to increased carbon flux through the pathway, they accumulate in significant amounts. The pathway intermediates accumulation is highly significant when the

truncated from of HMGR is overexpressed as compared to the full length form of HMGR suggesting that the overexpression of the truncated form of HMGR creates even greater increase in carbon flux through the pathway. This provides further evidence for additional control points for sterol biosynthesis in plants such as squalene epoxidase, sterol methyltransferase I, sterol C4-demethylase, obtusifoliol C14.alpha.-demethylase, sterol C5-desaturase, and sterol methyl transferase II.

US-PAT-NO: 6787683

DOCUMENT-IDENTIFIER: US 6787683 B1
See image for Certificate of Correction

TITLE: Phytyl/prenyltransferase nucleic acids, polypeptides and uses thereof

DATE-ISSUED: September 7, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Penna; Dean Della	Williamston	MI	N/A	N/A
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APPL-NO: 09/ 560761

DATE FILED: April 28, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a CIP of U.S. patent application Ser. No. 09/307,460 filed May 7, 1999, now abandoned which is hereby incorporated by reference.

US-CL-CURRENT: 800/281, 435/468 , 800/278

ABSTRACT:

The invention provides isolated nucleic acids and their encoded proteins that are involved in tocopherol or plastoquinone biosynthesis. The invention further provides recombinant expression cassettes, host cells, transgenic plants, and antibody compositions. The present invention provides methods and compositions relating to altering phytyl/prenyltransferase protein content and/or composition of plants.

3 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Brief Summary Text - BSTX (78):

The vector comprising the sequences from a polynucleotide of the present invention will typically comprise a marker gene which confers a selectable phenotype on plant cells. Usually, the selectable marker gene will encode antibiotic or herbicide resistance. Suitable genes include those coding for resistance to the antibiotic spectinomycin or streptomycin (e.g., the aada gene), the streptomycin **phosphotransferase** (SPT) gene coding for streptomycin resistance, the neomycin **phosphotransferase** (NPTII) gene encoding kanamycin or geneticin resistance, the hygromycin **phosphotransferase** (HPT) gene coding for hygromycin resistance.

Detailed Description Text - DETX (5):

To demonstrate that SLR1736 might be involved in tocopherol biosynthesis in Synechocystis, this gene was disrupted by insertion of the kanamycin expression cassette. The method of gene disruption by gene replacement technique has been previously described (Williams, 1988). The resulting mutant was named .DELTA.SLR1736. Before analyses, the mutant was sub-cultured at least 6 times by single colony section on kanamycin to select for the colonies containing only copies of the SLR1736 gene disrupted with the kanamycin resistance gene. The absence of wild type SLR1736 genes was confirmed by PCR. The lack of tocopherols in the mutant was shown by HPLC separation of lipid extracts from wild type and mutant Synechocystis on a normal-phase column using fluorescent detection (FLD). Levels of phylloquinone (vitamin K1) and plastoquinone seem to be unaffected in this mutant. This suggests that there are at least two separate prenyltransferase activities for tocopherol and plastoquinone synthesis in Synechocystis and we may be able to manipulate carbon flow through the pathway by altering gene expression of either. Phytylation/prenylation of homogentisic acid is the branch-point in tocopherol and plastoquinone synthesis, and therefore, most likely an important regulatory point of the pathway. As well as the prenylation activities, availability of different prenyl tails may also be crucial for the regulation of carbon flow through the pathway. This will become significant for manipulating tocopherol levels in higher plants.

Detailed Description Text - DETX (18):

The kanamycin resistance gene from the transposon Tn903 encoding aminoglycoside 3'-phosphotransferase was used to disrupt the wild type SLR1736 gene. Plasmid pUC4K (Pharmacia) was cut with EcoRI to release the kanamycin resistance expression cassette. Since SLR1736 has a unique MfeI site about 200 bp from the beginning of the gene, plasmid KS-1736 #5 was digested with MfeI (NEB). MfeI leaves 5'-cohesive ends compatible with EcoRI so that no other molecular manipulations are necessary. The two DNA fragments were purified from agarose gels as described above and ligated using T4 DNA ligase (Gibco BRL) as recommended by the manufacturer. Competent E. coli DH5.alpha. cells were transformed with the ligation reaction and transformants selected on LB plates containing 50 mg of kanamycin per liter of media. Plasmids were purified and subjected to restriction analysis. Two plasmids having opposite orientation of the kanamycin cassette were chosen for Synechocystis transformation. The two constructs were designated as KS.DELTA.1736-KAN-F and B, respectively, to indicate the orientation of the kanamycin resistance gene in respect to the SLR1736 gene.

Detailed Description Text - DETX (93):

A selectable marker gene which can be used to facilitate soybean transformation is a chimeric gene composed of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) Nature 313:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from E. coli; Gritz et al. (1983) Gene 25:179-188) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of Agrobacterium tumefaciens. The seed expression cassette comprising the phaseolin 5' region, the fragment encoding the instant polypeptides and the phaseolin 3' region can be isolated as a restriction fragment. this fragment can then be inserted into a unique restriction site of the vector carrying the marker gene.

US-PAT-NO: 6750049

DOCUMENT-IDENTIFIER: US 6750049 B1

TITLE: Synthesis of 1,2,3,4-tetrahydroxybenzenes and 1,2,3-trihydroxybenzenes using myo-inositol-1-phosphate synthase and myo-inositol 2-dehydrogenase

DATE-ISSUED: June 15, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Frost; John W.	Okemos	MI	N/A	N/A
Hansen; Chad A.	East Lansing	MI	N/A	N/A

APPL-NO: 09/ 937243

DATE FILED: May 7, 2002

PARENT-CASE:

RELATED APPLICATIONS

The present invention is a continuation-in-part of U.S. Ser. No. 09/274,732, filed Mar. 23, 1999, now abandoned which is hereby expressly incorporated by reference.

PCT-DATA:
APPL-NO: PCT/US00/06808
DATE-FILED: March 16, 2000
PUB-NO: WO00/56911
PUB-DATE: Sep 28, 2000
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/158, 435/190 , 435/233 , 435/252.3 , 435/252.33 , 435/252.4

ABSTRACT:

A bioengineered synthesis scheme for the production of 1,2,3,4-tetrahydroxybenzene from a carbon source is provided. Methods of producing 1,2,3,4-tetrahydroxybenzene from a carbon source based on the synthesis scheme are also provided. Methods are also provided for converting 1,2,3,4-tetrahydroxybenzene to 1,2,3-trihydroxybenzene by catalytic hydrogenation.

41 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

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Detailed Description Text - DETX (8):

In one embodiment, a recombinant E. coli microbe is employed in the methods of the present invention. In a preferred embodiment, the E. coli comprises a non-functional serA locus. This recombinant E. coli, designated, JWF1, may further comprise a plasmid carrying an INO1 gene insert and a serA gene insert. The INO1 gene encodes myo-inositol-1-phosphate synthase which converts glucose-6-phosphate to myo-inositol-1-phosphate. In a preferred embodiment, the INO1 gene is from Saccharomyces cerevisiae. Overexpression of myo-inositol-1-phosphate synthase will increase carbon flow into the myo-inositol pathway. This recombinant microbe is capable of converting glucose to myo-inositol.

Detailed Description Text - DETX (10):

In a preferred embodiment, the recombinant E. coli comprises plasmid pAD1.88A carrying an INO1 gene insert and a serA gene insert. As described above, the INO1 gene insert encodes myo-inositol-1-phosphate synthase which converts glucose-6-phosphate to myo-inositol-1-phosphate, thus increasing the carbon flow into the myo-inositol pathway. Due to a mutation in the E. coli genomic serA locus required for L-serine biosynthesis, growth in minimal salts medium and plasmid maintenance follows from expression of plasmid-localized serA. The serA insert thus allows microbial growth in minimal salts medium, distinguishing the microbes containing the plasmid from non-plasmid containing microbes.

Detailed Description Text - DETX (26):

Synthesis of myo-inositol by E. coli JWF1/pAD1.88A begins with D-glucose uptake and conversion to D-glucose-6-phosphate catalyzed by the E. coli phosphotransferase system (Postma, P. W. et al., In Escherichia coli and Salmonella, 2nd ed., Neidhardt, F. C. et al., Eds., ASM: Washington, Vol. 1, p. 1149 (1996)) where phosphoenolpyruvate is the source of the transferred phosphoryl group. D-Glucose-6-phosphate then undergoes cyclization to myo-inositol 1-phosphate catalyzed by myo-inositol-1-phosphate synthase. This enzyme activity, which results from expression of the Saccharomyces cerevisiae INO1 gene (Dean-Johnson, M. et al., J. Biol. Chem. 264:1274 11989)) on plasmid pAD1.88A, varied significantly (0.022, 0.043, 0.018, and 0.009 .mu.mol/min/mg at 18 h, 30 h, 42 h, and 54 h, respectively) over the course of the fermentation.

US-PAT-NO: 6716474

DOCUMENT-IDENTIFIER: US 6716474 B2

TITLE: Expression of fructose 1,6 bisphosphate aldolase in transgenic plants

DATE-ISSUED: April 6, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Barry; Gerard F.	St. Louis	MO	N/A	N/A
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APPL-NO: 09/ 923109

DATE FILED: August 6, 2001

PARENT-CASE:

This application is a divisional application Ser. No. 09/098,219 filed Jun. 16, 1998, now U.S. Pat. No. 6,441,277, which is based on U.S. Provisional application Serial No. 60/049,995, filed Jun. 17, 1997.

US-CL-CURRENT: 426/637

ABSTRACT:

Fructose-1,6-bisphosphate aldolase (FDA) is an enzyme reversibly catalyzing the reaction converting triosephosphate into fructose-1,6-bisphosphate. In the leaf, this enzyme is located in the chloroplast (starch synthesis) and the cytosol (sucrose biosynthesis). Transgenic plants were generated that express the E. coli fda gene in the chloroplast to improve plant yield by increasing leaf starch biosynthetic ability in particular and sucrose production in general. Leaves from plants expressing the fda transgene showed a significantly higher starch accumulation, as compared to control plants expressing the null vector, particularly early in the photoperiod, but had lower leaf sucrose. Transgenic plants also had a significantly higher root mass. Furthermore, transgenic potatoes expressing fda exhibited improved uniformity of solids.

2 Claims, 12 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 11

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Brief Summary Text - BSTX (17):

First, increasing the expression of the FDA enzyme in the chloroplast would increase the flow of carbon through the Calvin Cycle and increase atmospheric carbon assimilation during early photoperiod. This would result in an increase in photosynthetic efficiency and an increase in chloroplast starch production (a leaf carbon storage form degraded during periods when photosynthesis is low

or absent). Both of these responses would lead to an increase in sucrose production by the leaf and a net increase in carbon export during a given photoperiod. This increase in source capacity is a desirable trait in crop plants and would lead to increased plant growth, storage ability, yield, vigor, and stress tolerance.

Brief Summary Text - BSTX (19):

Third, expression of FDA in sink tissues can show several desirable traits, such as increased amino acid and/or fatty acid pools via increases in carbon flux through glycolysis (and thus pyruvate levels) in seeds or other sinks and increased starch levels as result of increased production of glucose 6-phosphate in seeds, roots, stems, and tubers where starch is a major storage nonstructural carbohydrate (reverse glycolysis). This increase in sink strength is a desirable trait in crop plants and would lead to increased plant growth, storage ability, yield, vigor, and stress tolerance.

Detailed Description Text - DETX (2):

This invention is directed to a method for producing plant cells and plants demonstrating an increased or improved growth and development, yield, quality, starch storage uniformity, vigor, and/or stress tolerance. The method utilizes a DNA sequence encoding an fda (fructose 1,6 bisphosphate aldolase) gene integrated in the cellular genome of a plant as the result of genetic engineering and causes expression of the FDA enzyme in the transgenic plant so produced. Plants that overexpress the FDA enzyme exhibit increased carbon flow through the Calvin Cycle and increased atmospheric carbon assimilation during early photoperiod resulting in an increase in photosynthetic efficiency and an increase in starch production. Thus, such plants exhibit higher levels of sucrose production by the leaf and the ability to achieve a net increase in carbon export during a given photoperiod. This increase in source capacity leads to increased plant growth that in turn generates greater biomass and/or increases the size of the sink and ultimately providing greater yields of the transgenic plant. This greater biomass or increased sink size may be evidenced in different ways or plant parts depending on the particular plant species or growing conditions of the plant overexpressing the FDA enzyme. Thus, increased size resulting from overexpression of FDA may be seen in the seed, fruit, stem, leaf, tuber, bulb or other plant part depending upon the plant species and its dominant sink during a particular growth phase and upon the environmental effects caused by certain growing conditions, e.g. drought, temperature or other stresses. Transgenic plants overexpressing FDA may therefore have increased carbon assimilation, export and storage in plant source and sink organs, which results in growth, yield, and uniformity and quality improvements.

Detailed Description Text - DETX (3):

Plants overexpressing FDA may also exhibit desirable quality traits such as increased production of starch, oils and/or proteins depending upon the plant species overexpressing the FDA. Thus, overexpression of FDA in a particular plant species may affect or alter the direction of the carbon flux thereby directing metabolite utilization and storage either to starch production, protein production or oil production via the role of FDA in the glycolysis and gluconeogenesis metabolic pathways.

Detailed Description Text - DETX (36):

A recombinant DNA molecule of the invention typically includes a selectable marker so that transformed cells can be easily identified and selected from non-transformed cells. Examples of such include, but are not limited to, a neomycin phosphotransferase (nptII) gene (Potrykus et al., 1985), which confers kanamycin resistance. Cells expressing the nptII gene can be selected using an appropriate antibiotic such as kanamycin or G418. Other commonly used

selectable markers include the bar gene, which confers bialaphos resistance; a mutant EPSP synthase gene (Hinchee et al., 1988), which confers glyphosate resistance; a nitrilase gene, which confers resistance to bromoxynil (Stalker et al., 1988); a mutant acetolactate synthase gene (ALS), which confers imidazolinone or sulphonylurea resistance (European Patent Application 154,204, 1985); and a methotrexate resistant DHFR gene (Thillet et al., 1988).

Detailed Description Text - DETX (88):
This increase in sucrose export by fda-expressing leaves is an illustration of an increase in source capacity, very likely due to an increased carbon flow through the Calvin Cycle (in response to increased triose-P utilization) and thus an increase in net carbon utilization by the leaf. As seen in Table 2, the increase in sucrose loading in the phloem correlates with the level of fda expression.

Detailed Description Text - DETX (94):
For the cytosolic expression of the fda gene in corn plants, a construct was made in which the fda gene sequence was fused to the backbone of a vector containing the enhanced CaMV 35S promoter (e35S; Kay et al., 1987), the HSP70 intron (U.S. Pat. No. 5,593,874), and the NOS3' polyadenylation sequence (Fraley et al., 1983). This created a NotI cassette [P-e35S/HSP70 intron/fda/NOS3'] that was cloned into the NotI site of pMON30460, a monocot transformation vector, to form the plant transformation vector pMON13925, as shown in FIG. 5. pMON30460 contains an expression cassette for the selectable marker neomycin phosphotransferase typeII gene (nptII) [P-35S/NPTII/NOS3'] and a unique NotI site for cloning the gene of interest. The final vector (pMON13925) was constructed so that the gene of interest and the selectable marker gene were cloned in the same orientation. A vector fragment containing the expression cassettes for these gene sequences could be excised from the bacterial selector (Kan) and ori, gel purified, and used for plant transformation.

Detailed Description Text - DETX (98):
Transgenic maize plants transformed with the vectors pMON13925 (described above) or pMON17590 (described above) were produced using microprojectile bombardment, a procedure well-known to the art (Fromm, 1990; Gordon-Kamm et al., 1990; Walters et al., 1992). Embryogenic callus initiated from immature maize embryos was used as a target tissue. Plasmid DNA at 1 mg/mL in TE buffer was precipitated onto M10 tungsten particles using a calcium chloride/spermidine procedure, essentially as described by Klein et al. (1988). In addition to the gene of interest, the plasmids also contained the neomycin phosphotransferase II gene (nptII) driven by the 35S promoter from Cauliflower Mosaic Virus. The embryogenic callus target tissue was pretreated on culture medium osmotically buffered with 0.2M mannitol plus 0.2M sorbitol for approximately four hours prior to bombardment (Vain et al., 1993). Tissue was bombarded two times with the DNA-coated tungsten particles using the gunpowder version of the BioRad Particle Delivery System (PDS) 1000 device. Approximately 16 hours following bombardment, the tissue was subcultured onto a medium of the same composition except that it contained no mannitol or sorbitol, and it contained an appropriate aminoglycoside antibiotic, such as G418", to select for those cells that contained and expressed the 35S/nptII gene. Actively growing tissue sectors were transferred to fresh selective medium approximately every 3 weeks. About 3 months after bombardment, plants were regenerated from surviving embryogenic callus essentially as described by Duncan and Widholm (1988).

Detailed Description Text - DETX (113):
A second potato transformation vector was constructed by cloning the NotI cassette [P-FMV/CTP2/fda/NOS3'] (described earlier) into the unique NotI site

of pMON23616. pMON23616 is a potato transformation vector containing the nopaline-type T-DNA right border region (Fraley et al., 1985), an expression cassette for the neomycin phosphotransferase typeII gene [P-35S/NPTII/NOS3'] (selectable marker), a unique NotI site for cloning the gene expression cassette of interest, and the T-DNA left border region (Barker et al., 1983). Cloning of the NotI cassette [P-FMV/CTP2/fda/NOS3'] (described earlier) into the NotI site of pMON23616 results in the potato transformation vector pMON17581, as shown in FIG. 8. The vector pMON 17581 was constructed such that the gene of interest and the selectable marker gene were transcribed in the same direction.

US-PAT-NO: 6682918

DOCUMENT-IDENTIFIER: US 6682918 B1

TITLE: Bacterial sucrose synthase compositions and methods of use

DATE-ISSUED: January 27, 2004

INVENTOR-INFORMATION:

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APPL-NO: 08/ 684005

DATE FILED: July 19, 1996

US-CL-CURRENT: 435/193

ABSTRACT:

The present invention provides isolated and purified polynucleotides that encode bacterial polypeptides that participate in the utilization of sucrose. Isolated bacterial sucrose synthase compositions and methods of use are provided. Processes for altering sucrose synthase activity, altering the starch and/or sucrose content of bacterial and/or plant cells, methods of identifying sucrose synthase-encoding nucleic acid segments, and compositions comprising sucrose synthase peptides and antibodies are also disclosed.

48 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

---------- KWIC ---------

Detailed Description Text - DETX (32):

In preferred embodiments, the vector used to express the polypeptide includes a selection marker that is effective in a plant cell, preferably a drug resistance selection marker. One preferred drug resistance marker is the gene whose expression results in kanamycin (Kan) resistance; i.e., the chimeric gene containing the nopaline synthase promoter, Tn5 neomycin **phosphotransferase** II (nptII) and nopaline synthase 3' nontranslated region described (Rogers et al., 1988).

Detailed Description Text - DETX (177):

An alternate plant transformation protocol utilizes the biolistic mechanism, whereby tungsten beads are coated with DNA and used to bombard plant tissue. In this protocol, the sucA gene may be engineered with specific plant promoter and processing sequences and cloned into a vector containing either a chimeric uidA gene encoding .beta.-glucuronidase or a chimeric kan gene encoding neomycin **phosphotransferase,** such as pFF19G or pFF19K (Maliga, 1995). This DNA is grown in E. coli and used to coat tungsten particles. The particles are

loaded into a biolistic gun, such as the PDS-1000/He (DuPont) and used to bombard plant tissues, such as embryos, protoplasts or leaves. The plant tissue may then be cultured on selective media and the resultant plant shoots regenerated on appropriate media.

Detailed Description Text - DETX (190):

The inventors contemplate that transformation of plants with the Anabaena sucA gene appropriately expressed via tissue-specific promoters is useful in the preparation of cultivars with improved features such as enhanced starch production in potato tubers and seed grains. The Anabaena sucA gene may be fused to specific promoters or promoter elements that will allow expression of sucrose synthase in a whole plant, tissue-specific, or subcellular compartment-specific manner. Many useful promoters and promoter elements are known that are suitable. One such promoter would be the patatin promoter that is active mainly in potato tubers (Bevan et al., 1986). The fusion of the Anabaena sucA gene to the patatin promoter and expression in plants may permit an increase in the expression of sucrose synthase activity in the tuber, possibly resulting in an increase in net carbon flow into the tuber and increased starch synthesis.

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	5111	carbon near2 (flux or flow)	US-PGPUB; USPAT	OR	OFF	2005/05/13 15:33
L2	374	1 near4 (modif$8 or alter$8 or increas$8)	US-PGPUB; USPAT	OR	OFF	2005/05/13 15:33
L3	233	(phosphoenol adj pyruvate or pep or phosphoenolpyruvate or phospho adj enol adj pyruvate) near4 (suppl$4 or availab$8)	US-PGPUB; USPAT	OR	OFF	2005/05/13 15:34
L4	11	2 and 3	US-PGPUB; USPAT	OR	OFF	2005/05/13 15:34
L5	8702	phosphotransferase$1 or phospho adj transferase$1	US-PGPUB; USPAT	OR	OFF	2005/05/13 15:51
L6	58	(2 or 3) and 5	US-PGPUB; USPAT	ADJ	OFF	2005/05/13 15:51
L7	43	(2 or 3) same (aromatic or shikimate)	US-PGPUB; USPAT	OR	OFF	2005/05/13 16:07

PGPUB-DOCUMENT-NUMBER: 20050079617

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20050079617 A1

TITLE: Glucose transport mutants for production of biomaterial

PUBLICATION-DATE: April 14, 2005

INVENTOR-INFORMATION:

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APPL-NO: 10/ 728337

DATE FILED: December 3, 2003

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
WO	PCT/US03/31544	2003WO-PCT/US03/31544	October 3, 2003

US-CL-CURRENT: 435/471

ABSTRACT:

A method is disclosed for restoring a Glu.sup.+ phenotype to a PTS.sup.-/Glu.sup.- bacterial cell which was originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport. Bacterial cells comprising the Glu.sup.+ phenotype have modified endogenous chromosomal regulatory regions which are operably linked to polynucleotides encoding galactose permeases and glucokinases.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to application PCT/US03/31544, filed Oct. 3, 2003, U.S. Provisional Application 60/416,166 filed Oct. 4, 2002 and U.S. Provisional Application 60/374,931 filed Oct. 4, 2002, which are hereby incorporated by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (19):

[0017] In a first aspect, the invention pertains to a method of increasing carbon flow into a metabolic pathway of a PTS.sup.-/Glu.sup.- bacterial host cell which was originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport which comprises a) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a glucose assimilation protein in a PTS.sup.-/Glu.sup.- host cell by transforming the PTS.sup.-/Glu.sup.- host cell with a DNA construct comprising a promoter and DNA flanking sequences corresponding to upstream (5') regions of the glucose assimilation protein; b) allowing integration of the DNA construct to restore a Glu+ phenotype; and c) culturing the transformed host cell under suitable culture conditions, wherein the carbon flow into a

metabolic pathway of the transformed host cell is increased compared to the carbon flow into the same metabolic pathway in a corresponding PTS bacterial host cell cultured under essentially the same culture conditions. In one embodiment of the method the promoter is a non-host cell promoter or a modified endogenous promoter. In a second embodiment the glucose assimilation protein is a glucose transporter, preferably a galactose permease obtained from E. coli or a glucose transporter having at least 80% sequence identity thereto. In a third embodiment the glucose assimilation protein is a phosphorylating protein, preferably a glucokinase obtained from E. coli or a glucokinase having at least 80% sequence identity thereto. In a fourth embodiment of the method the bacterial host cell is selected from the group consisting of E. coli cells, Bacillus cells and Pantoea cells. In a fifth embodiment, the PTS.sup.-/Glu.sup.- host cell is obtained from a PTS cell by deletion of one or more genes selected from the group consisting of ptsI, ptsH and crr. In a sixth embodiment, the PTS.sup.-/Glu.sup.+ host cell is transformed with a polynucleotide encoding a protein selected from the group consisting of a transketolase, a transaldolase, a phosphoenolpyruvate synthase, DAHP synthase, DHQ synthase, DHQ dehydratase, shikimate dehydrogenase, shikimate kinase EPSP synthase and chorismate synthase.

Summary of Invention Paragraph - BSTX (22):

[0020] In a fourth aspect, the invention pertains to a method of increasing carbon flow into a metabolic pathway of a PTS.sup.-/Glu.sup.- bacterial host cell originally capable of utilizing a phosphotransferase transport system (PTS) for carbohydrate transport which comprises a) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a galactose permease in a PTS.sup.-/Glu.sup.- host cell by transforming the PTS.sup.-/Glu.sup.- host cell with a first DNA construct comprising a promoter and DNA flanking sequences corresponding to upstream (5') regions of the galactose permease; b) modifying an endogenous chromosomal regulatory region which is operably linked to a nucleic acid encoding a glucokinase in the PTS.sup.-/Glu.sup.- host cell by transforming the PTS.sup.-/Glu.sup.- host cell with a second DNA construct comprising a promoter and DNA flanking sequences corresponding to upstream (5') regions of the glucokinase; c) allowing integration of the first and the second DNA constructs, wherein the first DNA construct replaces an endogenous promoter of the nucleic acid encoding the galactose permease and the second DNA construct replaces an endogenous promoter of the nucleic acid encoding the glucokinase wherein both the galactose permease and the glucokinase are expressed in the host cell and wherein said expression results in an increase in carbon flow into a metabolic pathway of the transformed host cell compared to carbon flow into the same metabolic pathway in the corresponding unaltered PTS.sup.-/Glu.sup.- bacterial cell. In one embodiment the metabolic pathway is the common aromatic pathway. In a second embodiment the method further comprises transforming the PTS.sup.-/Glu.sup.- host cell with a polynucleotide encoding a protein selected from the group consisting of a transketolase, a transaldolase and a phosphoenolpyruvate synthase.

Brief Description of Drawings Paragraph - DRTX (3):

[0027] The following abbreviations are used in the figure and throughout the disclosure: PEP=phosphoenolpyruvate; DAHP=3-deoxy-D-arabino-heptuloso- nate 7-phosphate; DHQ=3-dehydroquinate; DHS=3-dehydroshikimate; SHK=shikimate; S3P=shikimate 3-phosphate; EPSP=5-enolpyruvyl shikimate 3-phosphate; PHE=phenylalanine; TYR=tyrosine; TRP=tryptophan; Pyk=pyruvate kinase, which is encoded by the gene pyk; and Ppc=PEP carboxylase, which is encoded by the gene ppc. Further, the following genes are illustrated for the common aromatic pathway: aroB which encodes DHQ synthase; aroD which encodes DHQ dehydratase; aroE which encodes shikimate dehydrogenase; aroL and aroK which encode

shikimate kinase; aroA which encodes EPSP synthase and aroC which encodes chorismate synthase. While not specifically illustrated, one skilled in the art is aware that aroG, aroF and aroH encode the three isozymes of DAHP synthase which catalyzes the conversion of Erythrose-4P (E4P) and PEP to DAHP in E. coli. FIG. 1B illustrates the varied compounds, the production of which, may be enhanced by an increase in carbon flux and PEP availability according to the methods encompassed by the invention.

Detail Description Paragraph - DETX (124):

[0162] It should be noted that as the host cell is cultured in conditions which create an increase in carbon flow into the aromatic pathway, it may be necessary to identify and overcome rate-limiting steps in the pathway. This methodology is available to the artisan, see, for example, U.S. Pat. Nos. 5,168,056 and 5,776,736.

Detail Description Paragraph - DETX (127):

[0165] In addition to increasing the carbon flux through the aromatic pathway, the following genes may be overexpressed in PTS.sup.-/Glu.sup.+ cells according to the invention: pps which encodes PEP synthase in E. coli (see U.S. Pat. No. 5,985,617) and talA which encodes transaldolase (Iida et al. (1993) J. Bacterial. 175:5375-5383). Further any gene encoding an enzyme that catalyzes reactions within the common aromatic pathway (for example, DAHP synthase (aroF, aroG, aroH), DHQ synthase (aroB), DHQ dehydratase (aroD), shikimate dehydrogenase (aroE), shikimate kinase (aroL, aroK), EPSP synthase (aroA) and chorismate synthase (aroC) may be amplified in the PTS.sup.-/Glu.sup.+ cells encompassed by the present invention.

Detail Description Paragraph - DETX (135):

[0173] Thus, having provided a PTS.sup.-/Glu.sup.+ strain which conserves PEP resulting in an increase in carbon flux into a metabolic pathway, such as the aromatic amino acid pathway, glycolysis, the TCA cycle, and the pentose phosphate pathway, by redirecting PEP and PEP precursors, the inventors have provided a host system which can be utilized for enhanced production of desired compounds in comparison to the production of the same compounds in a corresponding PTS host cell.

PGPUB-DOCUMENT-NUMBER: 20040261147

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040261147 A1

TITLE: High level production of arbutin in green plants and microbes

PUBLICATION-DATE: December 23, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Meyer, Knut	Wilmington	DE	US	
Viitanen, Paul V.	West Chester	PA	US	
Flint, Dennis	Newark	DE	US	

APPL-NO: 10/ 462162

DATE FILED: June 16, 2003

US-CL-CURRENT: 800/278, 435/468 , 435/471 , 435/488

ABSTRACT:

This invention relates to methods and materials to produce hydroquinone glucoside in genetically modified green plants and microorganisms.

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Detail Description Paragraph - DETX (71):

[0092] All co-factors, co-enzymes and co-substrates required for these pathways (such as FAD, NADH, O.sub.2, and UDPG) are present in the E. coli cytoplasm. E. coli strains with elevated levels of chorismate resulting from increased flux of carbon into the shikimate pathway have been disclosed (Berry et al., Trends Biotech, 14(7):250-256 (1996); Bongaerts et al., Metabolic Engineering, 3(4):289-300 (2001); Tatarko and Romeo T., Curr. Microbiol., 43(1):26-32 (2001); U.S. Pat. No. 6,210,937 B1; U.S. Pat. No. 5,776,736 A; and WO 73484 A1) and can be used to produce arbutin from glucose using the molecular tools described below.

Detail Description Paragraph - DETX (147):

[0167] The molecular identification and isolation of the pHBA 1-hydroxylase gene described by Applicants also enables arbutin production in microbial systems such as E. coli (described in detail in Examples 7 and 8). The pHBA 1-hydroxylase gene is expressed in E. coli cells in combination with a chorismate pyruvate-lyase gene and a suitable glucosyltransferase gene (Examples 6 and 8). Expressing these three enzymes in E. coli provides a route to arbutin from a cheap, fermentable carbon source, such as glucose, by creating a three-step pathway from chorismate to arbutin (FIG. 1). Those skilled in the art will recognize that all cofactors, coenzymes and co-substrates required for this pathway such as FAD, NADH, O.sub.2, and UDP-glucose (UDPG) are present in the E. coli cytoplasm. Moreover E. coli strains with elevated levels of chorismate resulting from increased flux of carbon into the shikimate pathway have been disclosed (Berry et al., Trends Biotech., 14(7):250-256 (1996); Bongaerts et al., Metabolic Engineering, 3(4):289-300 (2001); Tatarko and Romeo, Current Microbiology, 43(1):26-32

(2001); U.S. Pat. No. 6,210,937; US 5776736; and WO 73484 A1) and can be used to produce arbutin from glucose using the molecular tools described below.

US-PAT-NO: 6794164

DOCUMENT-IDENTIFIER: US 6794164 B2

TITLE: Process for the isolation of polyhydroxy cyclic carboxylic acids

DATE-ISSUED: September 21, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Malmberg; Mats	Lund	N/A	N/A	SE
Westrup; Brita	Lund	N/A	N/A	SE

APPL-NO: 10/ 041865

DATE FILED: January 7, 2002

US-CL-CURRENT: 435/136, 424/94.1 , 562/125 , 562/507 , 562/510

ABSTRACT:

This invention is directed to the use of crystallization acids, such as acetic, lactic and propionic acids, to obtain high purity polyhydroxyl cyclic carboxylic acids (PCCA) from low purity aqueous solutions. The preferred PCCA is shikimic acid and the preferred crystallization acid is acetic acid. The method according to the invention is particularly applicable to the isolation of shikimic acid from a fermentation broth.

16 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Detailed Description Text - DETX (14):

Yet another source of shikimic acid upon which the present invention can be conducted is obtained from fermentations wherein the carbon flow is increased into the shikimate pathway by combining metabolic manipulations during the fermentation. Further enhancement of the production of shikimic acid can be achieved through nutrient limitation. For example, the growth of microorganisms can be limited by limiting the cells' availability of aromatic amino acids (no exogenous source of aromatic amino acids exist in the growth medium). Alternatively, or in combination with the other techniques, shikimic acid production can be enhanced by feeding inorganic phosphate to the microorganisms at a growth limiting rate.

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  * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 16:23:28 ON 13 MAY 2005

=> fil .bec
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 16:23:35 ON 13 MAY 2005
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

11 FILES IN THE FILE LIST

=> s carbon(2a)(flux or flow)
FILE 'MEDLINE'
        211552 CARBON
         26515 FLUX
        351840 FLOW
L1         834 CARBON(2A)(FLUX OR FLOW)

FILE 'SCISEARCH'
        353976 CARBON
        135682 FLUX
        590741 FLOW
L2        3109 CARBON(2A)(FLUX OR FLOW)

FILE 'LIFESCI'
         48047 CARBON
         10791 FLUX
         50360 FLOW
L3        1108 CARBON(2A)(FLUX OR FLOW)

FILE 'BIOTECHDS'
         12023 CARBON
          1682 FLUX
         13895 FLOW
L4         235 CARBON(2A)(FLUX OR FLOW)

FILE 'BIOSIS'
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         47579 FLUX
        363374 FLOW
L5        3395 CARBON(2A)(FLUX OR FLOW)

FILE 'EMBASE'
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         28512 FLUX
        357323 FLOW
L6         860 CARBON(2A)(FLUX OR FLOW)

FILE 'HCAPLUS'
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        240791 FLUX
        786811 FLOW
L7        5491 CARBON(2A)(FLUX OR FLOW)

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         36645 FLUX
        166050 FLOW
L8         265 CARBON(2A)(FLUX OR FLOW)
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         64940 CARBON
         18659 FLUX
         98559 FLOW
L9        1298 CARBON(2A)(FLUX OR FLOW)

FILE 'BIOTECHNO'
         37961 CARBON
          7514 FLUX
         51787 FLOW
L10         541 CARBON(2A)(FLUX OR FLOW)

FILE 'WPIDS'
        343161 CARBON
         76396 FLUX
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L11        1341 CARBON(2A)(FLUX OR FLOW)

TOTAL FOR ALL FILES
L12       18477 CARBON(2A)(FLUX OR FLOW)

=> s l12(6a)(modif? or alter? or increas?)
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        663608 ALTER?
       1993446 INCREAS?
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FILE 'SCISEARCH'
        506078 MODIF?
        664601 ALTER?
       2041515 INCREAS?
L14         208 L2 (6A)(MODIF? OR ALTER? OR INCREAS?)

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         95034 MODIF?
        177896 ALTER?
        501087 INCREAS?
L15         108 L3 (6A)(MODIF? OR ALTER? OR INCREAS?)

FILE 'BIOTECHDS'
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         26976 ALTER?
         65234 INCREAS?
L16          46 L4 (6A)(MODIF? OR ALTER? OR INCREAS?)

FILE 'BIOSIS'
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        662880 ALTER?
       2110652 INCREAS?
L17         200 L5 (6A)(MODIF? OR ALTER? OR INCREAS?)

FILE 'EMBASE'
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        622098 ALTER?
       1865543 INCREAS?
L18          94 L6 (6A)(MODIF? OR ALTER? OR INCREAS?)

FILE 'HCAPLUS'
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       3827700 INCREAS?
L19         232 L7 (6A)(MODIF? OR ALTER? OR INCREAS?)
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         90985 ALTER?
        183123 INCREAS?
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FILE 'ESBIOBASE'
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        236575 ALTER?
        720067 INCREAS?
L21        119 L9 (6A)(MODIF? OR ALTER? OR INCREAS?)

FILE 'BIOTECHNO'
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        148127 ALTER?
        383544 INCREAS?
L22         69 L10(6A)(MODIF? OR ALTER? OR INCREAS?)

FILE 'WPIDS'
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        431842 ALTER?
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L23         57 L11(6A)(MODIF? OR ALTER? OR INCREAS?)

TOTAL FOR ALL FILES
L24       1244 L12(6A)(MODIF? OR ALTER? OR INCREAS?)

=> s phosphoenolpyruvate or (phospho enol or phosphoenol)(w)pyruvate or pep
FILE 'MEDLINE'
          6819 PHOSPHOENOLPYRUVATE
          4911 PHOSPHO
          1189 ENOL
            70 PHOSPHO ENOL
                 (PHOSPHO(W)ENOL)
           246 PHOSPHOENOL
         25213 PYRUVATE
           283 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
          3336 PEP
L25       9513 PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
               OR PEP

FILE 'SCISEARCH'
          6460 PHOSPHOENOLPYRUVATE
          4384 PHOSPHO
          8134 ENOL
            78 PHOSPHO ENOL
                 (PHOSPHO(W)ENOL)
           240 PHOSPHOENOL
         20485 PYRUVATE
           298 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
          3296 PEP
L26       9225 PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
               OR PEP

FILE 'LIFESCI'
          2287 PHOSPHOENOLPYRUVATE
          1637 "PHOSPHO"
           256 "ENOL"
            25 PHOSPHO ENOL
                 ("PHOSPHO"(W)"ENOL")
           119 PHOSPHOENOL
          6259 PYRUVATE
           132 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
          1012 PEP
```

```
L27        2971 PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
                OR PEP

FILE 'BIOTECHDS'
            503 PHOSPHOENOLPYRUVATE
            253 PHOSPHO
            170 ENOL
              6 PHOSPHO ENOL
                  (PHOSPHO(W)ENOL)
            131 PHOSPHOENOL
           2127 PYRUVATE
            133 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
            258 PEP
L28         769 PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
                OR PEP

FILE 'BIOSIS'
           8490 PHOSPHOENOLPYRUVATE
          58187 PHOSPHO
           2208 ENOL
            167 PHOSPHO ENOL
                  (PHOSPHO(W)ENOL)
           3680 PHOSPHOENOL
          36636 PYRUVATE
           3779 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
           4068 PEP
L29       12909 PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
                OR PEP

FILE 'EMBASE'
           4617 PHOSPHOENOLPYRUVATE
           3903 "PHOSPHO"
           2177 "ENOL"
             48 PHOSPHO ENOL
                  ("PHOSPHO"(W)"ENOL")
            189 PHOSPHOENOL
          20745 PYRUVATE
            218 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
           2893 PEP
L30        7008 PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
                OR PEP

FILE 'HCAPLUS'
          11382 PHOSPHOENOLPYRUVATE
           9949 PHOSPHO
          22228 ENOL
             67 PHOSPHO ENOL
                  (PHOSPHO(W)ENOL)
            728 PHOSPHOENOL
          49364 PYRUVATE
            702 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
           6683 PEP
L31       16639 PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
                OR PEP

FILE 'NTIS'
             37 PHOSPHOENOLPYRUVATE
             58 PHOSPHO
             77 ENOL
              0 PHOSPHO ENOL
                  (PHOSPHO(W)ENOL)
              5 PHOSPHOENOL
            306 PYRUVATE
              3 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
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```
          1354 PEP
L32       1386 PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
               OR PEP

FILE 'ESBIOBASE'
          2206 PHOSPHOENOLPYRUVATE
          2662 PHOSPHO
           517 ENOL
            29 PHOSPHO ENOL
                 (PHOSPHO(W)ENOL)
            93 PHOSPHOENOL
          6677 PYRUVATE
           119 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
          1161 PEP
L33       3039 PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
               OR PEP

FILE 'BIOTECHNO'
          2544 PHOSPHOENOLPYRUVATE
          1717 PHOSPHO
           192 ENOL
            21 PHOSPHO ENOL
                 (PHOSPHO(W)ENOL)
            82 PHOSPHOENOL
          6527 PYRUVATE
            96 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
           819 PEP
L34       3075 PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
               OR PEP

FILE 'WPIDS'
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          3629 PHOSPHO
          1803 ENOL
            79 PHOSPHO ENOL
                 (PHOSPHO(W)ENOL)
           199 PHOSPHOENOL
          2047 PYRUVATE
           205 (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
           448 PEP
L35        799 PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W)PYRUVATE
               OR PEP

TOTAL FOR ALL FILES
L36      67333 PHOSPHOENOLPYRUVATE OR (PHOSPHO ENOL OR PHOSPHOENOL)(W) PYRUVATE
                OR PEP

=> s l36(4a)(suppl#### or availab?)
FILE 'MEDLINE'
        365570 SUPPL####
        353142 AVAILAB?
L37         45 L25(4A)(SUPPL#### OR AVAILAB?)

FILE 'SCISEARCH'
        123739 SUPPL####
        395727 AVAILAB?
L38         53 L26(4A)(SUPPL#### OR AVAILAB?)

FILE 'LIFESCI'
         23230 SUPPL####
         92976 AVAILAB?
L39         27 L27(4A)(SUPPL#### OR AVAILAB?)

FILE 'BIOTECHDS'
```

```
          7569 SUPPL####
          9223 AVAILAB?
L40         15 L28(4A)(SUPPL#### OR AVAILAB?)

FILE 'BIOSIS'
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        300652 AVAILAB?
L41         42 L29(4A)(SUPPL#### OR AVAILAB?)

FILE 'EMBASE'
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        310156 AVAILAB?
L42         35 L30(4A)(SUPPL#### OR AVAILAB?)

FILE 'HCAPLUS'
        294096 SUPPL####
        433899 AVAILAB?
L43         72 L31(4A)(SUPPL#### OR AVAILAB?)

FILE 'NTIS'
         86949 SUPPL####
        169372 AVAILAB?
L44         16 L32(4A)(SUPPL#### OR AVAILAB?)

FILE 'ESBIOBASE'
         30675 SUPPL####
        118451 AVAILAB?
L45         21 L33(4A)(SUPPL#### OR AVAILAB?)

FILE 'BIOTECHNO'
         14397 SUPPL####
         58426 AVAILAB?
L46         17 L34(4A)(SUPPL#### OR AVAILAB?)

FILE 'WPIDS'
       1044324 SUPPL####
        114346 AVAILAB?
L47          8 L35(4A)(SUPPL#### OR AVAILAB?)

TOTAL FOR ALL FILES
L48        351 L36(4A)(SUPPL#### OR AVAILAB?)

=> s phosphotransferase# or phospho transferase#
FILE 'MEDLINE'
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          4911 PHOSPHO
         54262 TRANSFERASE#
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                 (PHOSPHO(W)TRANSFERASE#)
L49      20195 PHOSPHOTRANSFERASE# OR PHOSPHO TRANSFERASE#

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          4384 PHOSPHO
         42554 TRANSFERASE#
            18 PHOSPHO TRANSFERASE#
                 (PHOSPHO(W)TRANSFERASE#)
L50       5033 PHOSPHOTRANSFERASE# OR PHOSPHO TRANSFERASE#

FILE 'LIFESCI'
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          1637 "PHOSPHO"
         13552 TRANSFERASE#
             9 PHOSPHO TRANSFERASE#
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L51        3004 PHOSPHOTRANSFERASE# OR PHOSPHO TRANSFERASE#

FILE 'BIOTECHDS'
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           3455 TRANSFERASE#
              2 PHOSPHO TRANSFERASE#
                (PHOSPHO(W)TRANSFERASE#)
L52        2098 PHOSPHOTRANSFERASE# OR PHOSPHO TRANSFERASE#

FILE 'BIOSIS'
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          58187 PHOSPHO
          74049 TRANSFERASE#
           1758 PHOSPHO TRANSFERASE#
                (PHOSPHO(W)TRANSFERASE#)
L53        7582 PHOSPHOTRANSFERASE# OR PHOSPHO TRANSFERASE#

FILE 'EMBASE'
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           3903 "PHOSPHO"
          38606 TRANSFERASE#
              9 PHOSPHO TRANSFERASE#
                ("PHOSPHO"(W)TRANSFERASE#)
L54       12246 PHOSPHOTRANSFERASE# OR PHOSPHO TRANSFERASE#

FILE 'HCAPLUS'
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           9949 PHOSPHO
          50074 TRANSFERASE#
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                (PHOSPHO(W)TRANSFERASE#)
L55        8096 PHOSPHOTRANSFERASE# OR PHOSPHO TRANSFERASE#

FILE 'NTIS'
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             58 PHOSPHO
           1278 TRANSFERASE#
              0 PHOSPHO TRANSFERASE#
                (PHOSPHO(W)TRANSFERASE#)
L56         131 PHOSPHOTRANSFERASE# OR PHOSPHO TRANSFERASE#

FILE 'ESBIOBASE'
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           2662 PHOSPHO
          32718 TRANSFERASE#
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L57        3644 PHOSPHOTRANSFERASE# OR PHOSPHO TRANSFERASE#

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          16723 TRANSFERASE#
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L59         356 PHOSPHOTRANSFERASE# OR PHOSPHO TRANSFERASE#

TOTAL FOR ALL FILES
L60       69403 PHOSPHOTRANSFERASE# OR PHOSPHO TRANSFERASE#

=> s l12 and l60
FILE 'MEDLINE'
L61          25 L1 AND L49

FILE 'SCISEARCH'
L62          33 L2 AND L50

FILE 'LIFESCI'
L63          12 L3 AND L51

FILE 'BIOTECHDS'
L64           7 L4 AND L52

FILE 'BIOSIS'
L65          18 L5 AND L53

FILE 'EMBASE'
L66          13 L6 AND L54

FILE 'HCAPLUS'
L67          21 L7 AND L55

FILE 'NTIS'
L68           1 L8 AND L56

FILE 'ESBIOBASE'
L69          16 L9 AND L57

FILE 'BIOTECHNO'
L70          14 L10 AND L58

FILE 'WPIDS'
L71           2 L11 AND L59

TOTAL FOR ALL FILES
L72         162 L12 AND L60

=> s l60(8a)(delet? or inactivat?)
FILE 'MEDLINE'
        136235 DELET?
        106731 INACTIVAT?
L73        106 L49(8A)(DELET? OR INACTIVAT?)

FILE 'SCISEARCH'
        118498 DELET?
         90881 INACTIVAT?
L74         65 L50(8A)(DELET? OR INACTIVAT?)

FILE 'LIFESCI'
         59379 DELET?
         40828 INACTIVAT?
L75         76 L51(8A)(DELET? OR INACTIVAT?)

FILE 'BIOTECHDS'
         14166 DELET?
          8291 INACTIVAT?
L76         47 L52(8A)(DELET? OR INACTIVAT?)
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FILE 'BIOSIS'
        128579 DELET?
        113806 INACTIVAT?
L77        114 L53(8A)(DELET? OR INACTIVAT?)

FILE 'EMBASE'
        114134 DELET?
         93104 INACTIVAT?
L78         75 L54(8A)(DELET? OR INACTIVAT?)

FILE 'HCAPLUS'
        129189 DELET?
        143397 INACTIVAT?
L79        163 L55(8A)(DELET? OR INACTIVAT?)

FILE 'NTIS'
          4498 DELET?
          2164 INACTIVAT?
L80          0 L56(8A)(DELET? OR INACTIVAT?)

FILE 'ESBIOBASE'
         64611 DELET?
         41847 INACTIVAT?
L81         30 L57(8A)(DELET? OR INACTIVAT?)

FILE 'BIOTECHNO'
         73821 DELET?
         40724 INACTIVAT?
L82         58 L58(8A)(DELET? OR INACTIVAT?)

FILE 'WPIDS'
         29569 DELET?
         12585 INACTIVAT?
L83          6 L59(8A)(DELET? OR INACTIVAT?)

TOTAL FOR ALL FILES
L84        740 L60(8A)(DELET? OR INACTIVAT?)

=> s l84 and transport?
FILE 'MEDLINE'
        296421 TRANSPORT?
L85         11 L73 AND TRANSPORT?

FILE 'SCISEARCH'
        443238 TRANSPORT?
L86          9 L74 AND TRANSPORT?

FILE 'LIFESCI'
         72351 TRANSPORT?
L87          8 L75 AND TRANSPORT?

FILE 'BIOTECHDS'
          6416 TRANSPORT?
L88          5 L76 AND TRANSPORT?

FILE 'BIOSIS'
       2677074 TRANSPORT?
L89         15 L77 AND TRANSPORT?

FILE 'EMBASE'
        299979 TRANSPORT?
L90         13 L78 AND TRANSPORT?

FILE 'HCAPLUS'
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        727010 TRANSPORT?
L91          24 L79 AND TRANSPORT?

FILE 'NTIS'
        139160 TRANSPORT?
L92           0 L80 AND TRANSPORT?

FILE 'ESBIOBASE'
        200631 TRANSPORT?
L93           8 L81 AND TRANSPORT?

FILE 'BIOTECHNO'
         85418 TRANSPORT?
L94           9 L82 AND TRANSPORT?

FILE 'WPIDS'
        292993 TRANSPORT?
L95           1 L83 AND TRANSPORT?

TOTAL FOR ALL FILES
L96         103 L84 AND TRANSPORT?

=> s l60 and glucose
FILE 'MEDLINE'
        266672 GLUCOSE
L97        2512 L49 AND GLUCOSE

FILE 'SCISEARCH'
        192558 GLUCOSE
L98         928 L50 AND GLUCOSE

FILE 'LIFESCI'
         42406 GLUCOSE
L99         557 L51 AND GLUCOSE

FILE 'BIOTECHDS'
         31108 GLUCOSE
L100        138 L52 AND GLUCOSE

FILE 'BIOSIS'
        273610 GLUCOSE
L101       1252 L53 AND GLUCOSE

FILE 'EMBASE'
        223080 GLUCOSE
L102       1131 L54 AND GLUCOSE

FILE 'HCAPLUS'
        382557 GLUCOSE
L103       1511 L55 AND GLUCOSE

FILE 'NTIS'
          2876 GLUCOSE
L104          9 L56 AND GLUCOSE

FILE 'ESBIOBASE'
         65106 GLUCOSE
L105        492 L57 AND GLUCOSE

FILE 'BIOTECHNO'
         43289 GLUCOSE
L106        676 L58 AND GLUCOSE

FILE 'WPIDS'
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         32982 GLUCOSE
L107        56 L59 AND GLUCOSE

TOTAL FOR ALL FILES
L108      9262 L60 AND GLUCOSE

=> s l84 and l108
FILE 'MEDLINE'
L109        14 L73 AND L97

FILE 'SCISEARCH'
L110        10 L74 AND L98

FILE 'LIFESCI'
L111         9 L75 AND L99

FILE 'BIOTECHDS'
L112         7 L76 AND L100

FILE 'BIOSIS'
L113        16 L77 AND L101

FILE 'EMBASE'
L114        14 L78 AND L102

FILE 'HCAPLUS'
L115        27 L79 AND L103

FILE 'NTIS'
L116         0 L80 AND L104

FILE 'ESBIOBASE'
L117         6 L81 AND L105

FILE 'BIOTECHNO'
L118         9 L82 AND L106

FILE 'WPIDS'
L119         2 L83 AND L107

TOTAL FOR ALL FILES
L120       114 L84 AND L108

=> s l108 and transport
FILE 'MEDLINE'
        254687 TRANSPORT
L121       741 L97 AND TRANSPORT

FILE 'SCISEARCH'
        381436 TRANSPORT
L122       414 L98 AND TRANSPORT

FILE 'LIFESCI'
         56425 TRANSPORT
L123       226 L99 AND TRANSPORT

FILE 'BIOTECHDS'
          4481 TRANSPORT
L124        43 L100 AND TRANSPORT

FILE 'BIOSIS'
       2631403 TRANSPORT
L125       458 L101 AND TRANSPORT
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FILE 'EMBASE'
       267785 TRANSPORT
L126      493 L102 AND TRANSPORT

FILE 'HCAPLUS'
       650334 TRANSPORT
L127      641 L103 AND TRANSPORT

FILE 'NTIS'
        79193 TRANSPORT
L128        3 L104 AND TRANSPORT

FILE 'ESBIOBASE'
       181970 TRANSPORT
L129      244 L105 AND TRANSPORT

FILE 'BIOTECHNO'
        74784 TRANSPORT
L130      315 L106 AND TRANSPORT

FILE 'WPIDS'
       193572 TRANSPORT
L131       27 L107 AND TRANSPORT

TOTAL FOR ALL FILES
L132     3605 L108 AND TRANSPORT

=> s l132 and l36
FILE 'MEDLINE'
L133      403 L121 AND L25

FILE 'SCISEARCH'
L134      279 L122 AND L26

FILE 'LIFESCI'
L135      160 L123 AND L27

FILE 'BIOTECHDS'
L136       35 L124 AND L28

FILE 'BIOSIS'
L137      293 L125 AND L29

FILE 'EMBASE'
L138      284 L126 AND L30

FILE 'HCAPLUS'
L139      468 L127 AND L31

FILE 'NTIS'
L140        3 L128 AND L32

FILE 'ESBIOBASE'
L141      134 L129 AND L33

FILE 'BIOTECHNO'
L142      194 L130 AND L34

FILE 'WPIDS'
L143       25 L131 AND L35

TOTAL FOR ALL FILES
L144     2278 L132 AND L36
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=> s l144 and mut/q
FILE 'MEDLINE'
L145       252 L133 AND MUT/Q

FILE 'SCISEARCH'
L146       170 L134 AND MUT/Q

FILE 'LIFESCI'
L147        88 L135 AND MUT/Q

FILE 'BIOTECHDS'
L148        24 L136 AND MUT/Q

FILE 'BIOSIS'
L149       168 L137 AND MUT/Q

FILE 'EMBASE'
L150       162 L138 AND MUT/Q

FILE 'HCAPLUS'
L151       254 L139 AND MUT/Q

FILE 'NTIS'
L152         2 L140 AND MUT/Q

FILE 'ESBIOBASE'
L153        85 L141 AND MUT/Q

FILE 'BIOTECHNO'
L154       119 L142 AND MUT/Q

FILE 'WPIDS'
L155         6 L143 AND MUT/Q

TOTAL FOR ALL FILES
L156      1330 L144 AND MUT/Q

=> s l156 and (aromatic or shikimate)
FILE 'MEDLINE'
         33045 AROMATIC
           460 SHIKIMATE
L157         5 L145 AND (AROMATIC OR SHIKIMATE)

FILE 'SCISEARCH'
        102529 AROMATIC
           951 SHIKIMATE
L158         5 L146 AND (AROMATIC OR SHIKIMATE)

FILE 'LIFESCI'
         14691 AROMATIC
           306 SHIKIMATE
L159         2 L147 AND (AROMATIC OR SHIKIMATE)

FILE 'BIOTECHDS'
          5433 AROMATIC
           127 SHIKIMATE
L160         4 L148 AND (AROMATIC OR SHIKIMATE)

FILE 'BIOSIS'
         50696 AROMATIC
          1243 SHIKIMATE
L161         2 L149 AND (AROMATIC OR SHIKIMATE)

FILE 'EMBASE'
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         49121 AROMATIC
           345 SHIKIMATE
L162         3 L150 AND (AROMATIC OR SHIKIMATE)

FILE 'HCAPLUS'
        220110 AROMATIC
        298279 AROM
        416075 AROMATIC
                 (AROMATIC OR AROM)
          1906 SHIKIMATE
L163        10 L151 AND (AROMATIC OR SHIKIMATE)

FILE 'NTIS'
         11413 AROMATIC
             8 SHIKIMATE
L164         0 L152 AND (AROMATIC OR SHIKIMATE)

FILE 'ESBIOBASE'
         18251 AROMATIC
           347 SHIKIMATE
L165         3 L153 AND (AROMATIC OR SHIKIMATE)

FILE 'BIOTECHNO'
         12919 AROMATIC
           232 SHIKIMATE
L166         2 L154 AND (AROMATIC OR SHIKIMATE)

FILE 'WPIDS'
        185875 AROMATIC
          1766 AROM
        187038 AROMATIC
                 (AROMATIC OR AROM)
            69 SHIKIMATE
L167         5 L155 AND (AROMATIC OR SHIKIMATE)

TOTAL FOR ALL FILES
L168        41 L156 AND (AROMATIC OR SHIKIMATE)

=> s l24 and l36
FILE 'MEDLINE'
L169         9 L13 AND L25

FILE 'SCISEARCH'
L170        15 L14 AND L26

FILE 'LIFESCI'
L171        10 L15 AND L27

FILE 'BIOTECHDS'
L172         8 L16 AND L28

FILE 'BIOSIS'
L173        19 L17 AND L29

FILE 'EMBASE'
L174         8 L18 AND L30

FILE 'HCAPLUS'
L175        26 L19 AND L31

FILE 'NTIS'
L176         0 L20 AND L32

FILE 'ESBIOBASE'
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L177        10 L21 AND L33

FILE 'BIOTECHNO'
L178         9 L22 AND L34

FILE 'WPIDS'
L179         4 L23 AND L35

TOTAL FOR ALL FILES
L180       118 L24 AND L36

=> s l24 and glucose
FILE 'MEDLINE'
        266672 GLUCOSE
L181        39 L13 AND GLUCOSE

FILE 'SCISEARCH'
        192558 GLUCOSE
L182        39 L14 AND GLUCOSE

FILE 'LIFESCI'
         42406 GLUCOSE
L183        25 L15 AND GLUCOSE

FILE 'BIOTECHDS'
         31108 GLUCOSE
L184        21 L16 AND GLUCOSE

FILE 'BIOSIS'
        273610 GLUCOSE
L185        40 L17 AND GLUCOSE

FILE 'EMBASE'
        223080 GLUCOSE
L186        40 L18 AND GLUCOSE

FILE 'HCAPLUS'
        382557 GLUCOSE
L187        54 L19 AND GLUCOSE

FILE 'NTIS'
          2876 GLUCOSE
L188         0 L20 AND GLUCOSE

FILE 'ESBIOBASE'
         65106 GLUCOSE
L189        28 L21 AND GLUCOSE

FILE 'BIOTECHNO'
         43289 GLUCOSE
L190        30 L22 AND GLUCOSE

FILE 'WPIDS'
         32982 GLUCOSE
L191         8 L23 AND GLUCOSE

TOTAL FOR ALL FILES
L192       324 L24 AND GLUCOSE

=> s (148 or 172 or 196 or l120 or l156 or l168 or l180 or l192)
FILE 'MEDLINE'
L193       368 (L37 OR L61 OR L85 OR L109 OR L145 OR L157 OR L169 OR L181)

FILE 'SCISEARCH'
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L194       301 (L38 OR L62 OR L86 OR L110 OR L146 OR L158 OR L170 OR L182)

FILE 'LIFESCI'
L195       164 (L39 OR L63 OR L87 OR L111 OR L147 OR L159 OR L171 OR L183)

FILE 'BIOTECHDS'
L196        65 (L40 OR L64 OR L88 OR L112 OR L148 OR L160 OR L172 OR L184)

FILE 'BIOSIS'
L197       290 (L41 OR L65 OR L89 OR L113 OR L149 OR L161 OR L173 OR L185)

FILE 'EMBASE'
L198       259 (L42 OR L66 OR L90 OR L114 OR L150 OR L162 OR L174 OR L186)

FILE 'HCAPLUS'
L199       430 (L43 OR L67 OR L91 OR L115 OR L151 OR L163 OR L175 OR L187)

FILE 'NTIS'
L200        19 (L44 OR L68 OR L92 OR L116 OR L152 OR L164 OR L176 OR L188)

FILE 'ESBIOBASE'
L201       155 (L45 OR L69 OR L93 OR L117 OR L153 OR L165 OR L177 OR L189)

FILE 'BIOTECHNO'
L202       187 (L46 OR L70 OR L94 OR L118 OR L154 OR L166 OR L178 OR L190)

FILE 'WPIDS'
L203        19 (L47 OR L71 OR L95 OR L119 OR L155 OR L167 OR L179 OR L191)

TOTAL FOR ALL FILES
L204      2257 (L48 OR L72 OR L96 OR L120 OR L156 OR L168 OR L180 OR L192)

=> s l204 and py=<1995 range=2003,
FILE 'MEDLINE'
'2003,' IS NOT A VALID RANGE FOR FILE 'MEDLINE'
SEARCH ENDED BY USER

FILE 'SCISEARCH'
             0 PY=<1995
L205         0 L194 AND PY=<1995

FILE 'LIFESCI'
           120 PY=<1995
L206         0 L195 AND PY=<1995

FILE 'BIOTECHDS'
             6 PY=<1995
                 (PY=<1995)
L207         0 L196 AND PY=<1995

FILE 'BIOSIS'
            34 PY=<1995
L208         0 L197 AND PY=<1995

FILE 'EMBASE'
             5 PY=<1995
L209         0 L198 AND PY=<1995

FILE 'HCAPLUS'
           632 PY=<1995
L210         0 L199 AND PY=<1995

FILE 'NTIS'
           963 PY=<1995
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L211          0 L200 AND PY=<1995

FILE 'ESBIOBASE'
              0 PY=<1995
L212          0 L201 AND PY=<1995

FILE 'BIOTECHNO'
         845083 PY=<1995
L213         75 L202 AND PY=<1995

FILE 'WPIDS'
           4808 PY=<1995
                  (PY=<1995)
L214          0 L203 AND PY=<1995

TOTAL FOR ALL FILES
L215         75 L204 AND PY=<1995

=> fil medl
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                      40.76      40.97

FILE 'MEDLINE' ENTERED AT 16:38:40 ON 13 MAY 2005

 => s l204 and py=<1995 range=2003000000,
          11954 PY=<1995
L216          0 L193 AND PY=<1995

=> fil .becpat
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.38      41.35

FILES 'BIOTECHDS, HCAPLUS, WPIDS' ENTERED AT 16:39:18 ON 13 MAY 2005
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

3 FILES IN THE FILE LIST

=> s l204 and wo/pc and pry=<1995 range=2003,
FILE 'BIOTECHDS'
          17940 WO/PC
            236 PRY=<1995
                  (PRY=<1995)
L217          0 L196 AND WO/PC AND PRY=<1995

FILE 'HCAPLUS'
         129652 WO/PC
          10295 PRY=<1995
L218          0 L199 AND WO/PC AND PRY=<1995

FILE 'WPIDS'
         275190 WO/PC
          20949 PRY=<1995
                  (PRY=<1995)
L219          0 L203 AND WO/PC AND PRY=<1995

TOTAL FOR ALL FILES
L220          0 L204 AND WO/PC AND PRY=<1995

=> log y
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       7.97      49.32
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STN INTERNATIONAL LOGOFF AT 16:40:05 ON 13 MAY 2005